Figure 1

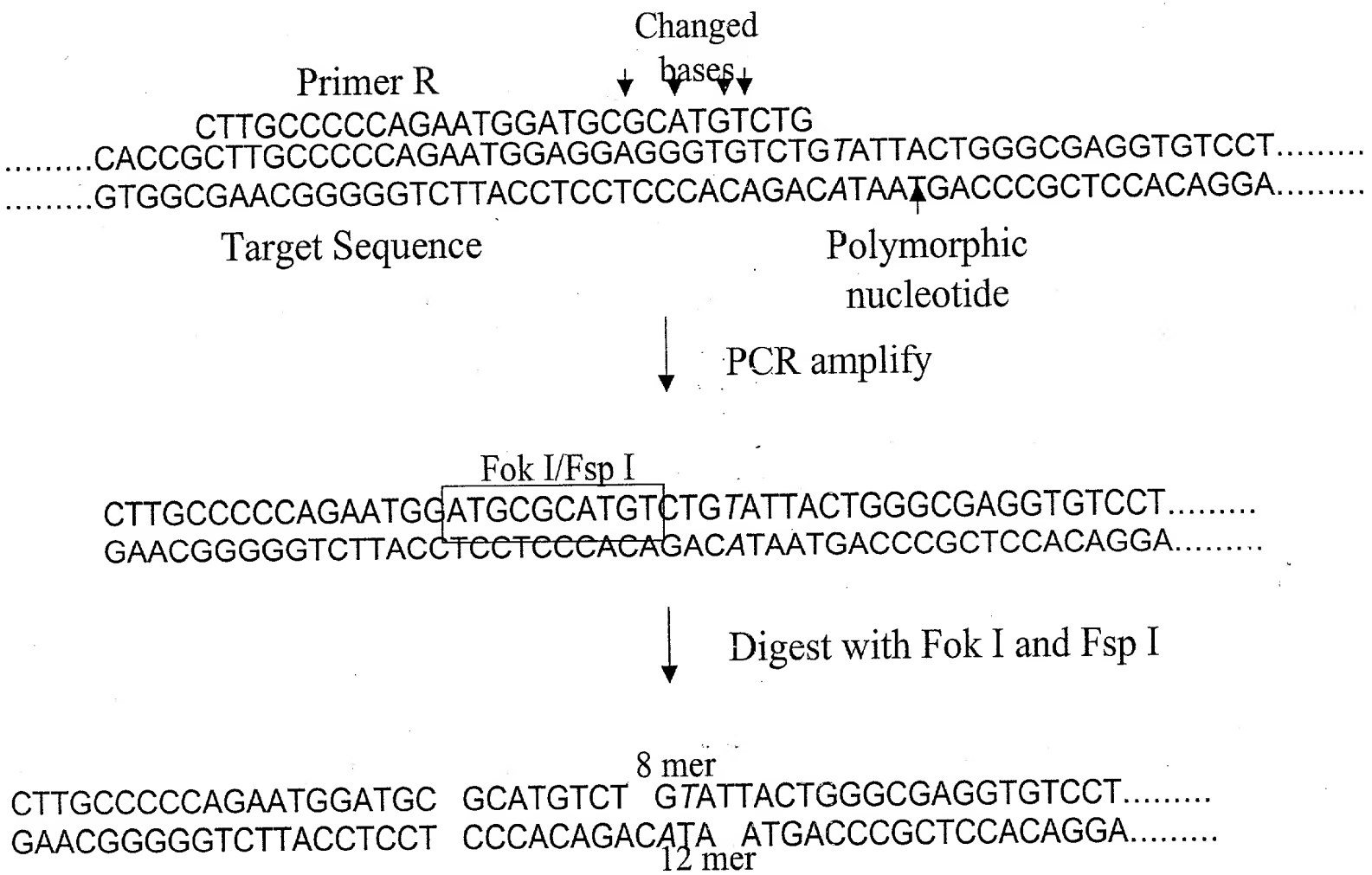

Changed
bases
Primer R
CTTGCCCCCAGAATGGATGCGCATGTCTG
.....CACCGCTTGCCCCCAGAATGGAGGAGGGTGTCTGTATTACTGGGCGAGGTGTCCT.....
.....GTGGCGAACGGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCACAGGA.....
Target Sequence
Polymorphic
nucleotide
PCR amplify
Fok I/Fsp I
CTTGCCCCCAGAATGGATGCGCATGTCTGTATTACTGGGCGAGGTGTCCT.....
GAACGGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCACAGGA.....
Digest with Fok I and Fsp I
8 mer
CTTGCCCCCAGAATGGATGC GCATGTCT GTATTACTGGGCGAGGTGTCCT.....
GAACGGGGGGTCTTACCTCCT CCCACAGACATA ATGACCCGCTCCACAGGA.....
12 mer

Figure 2

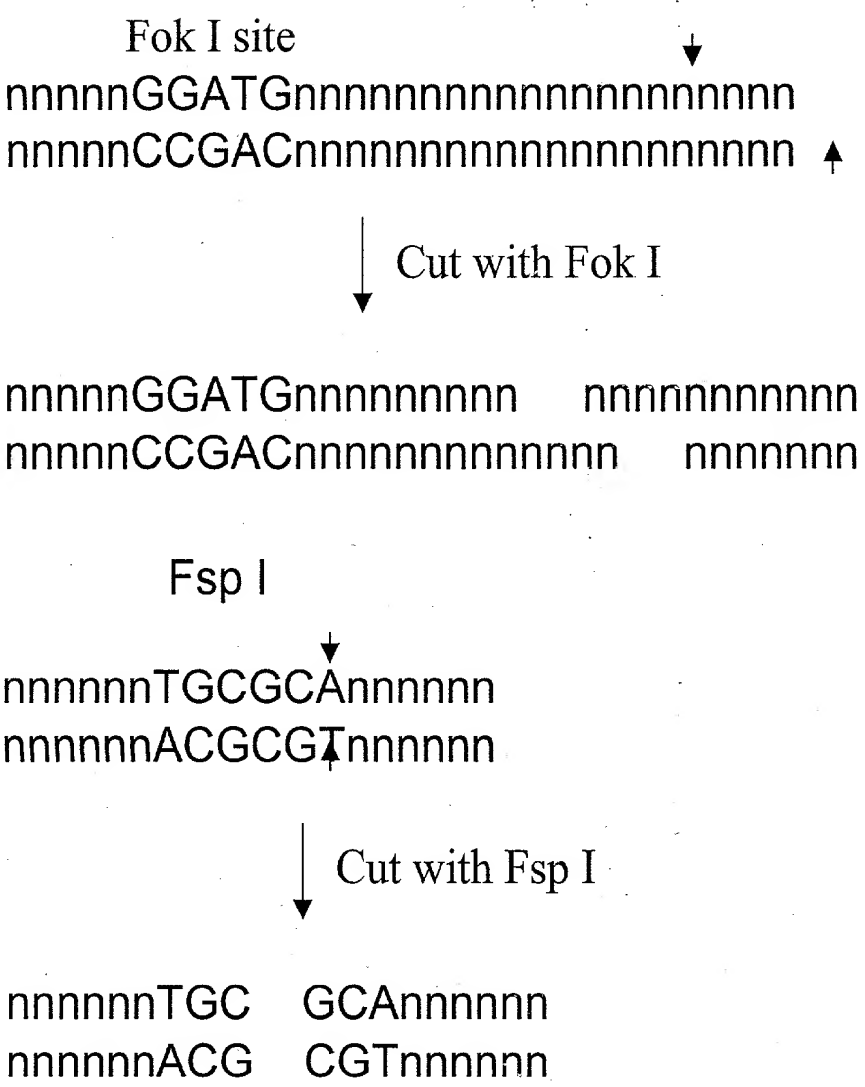

Fok I site
nnnnnGGATGnnnnnnnnnnnnnnnnnnn
nnnnnCCGACnnnnnnnnnnnnnnnnnnn
Cut with Fok I
nnnnnGGATGnnnnnnnnn nnnnnnnnnnn
nnnnnCCGACnnnnnnnnnnnnn nnnnnnn
Fsp I
nnnnnnTGCGCAnnnnnn
nnnnnnACGCGTnnnnnn
Cut with Fsp I
nnnnnnTGC GCAnnnnnn
nnnnnnACG CGTnnnnnn

Figure 3

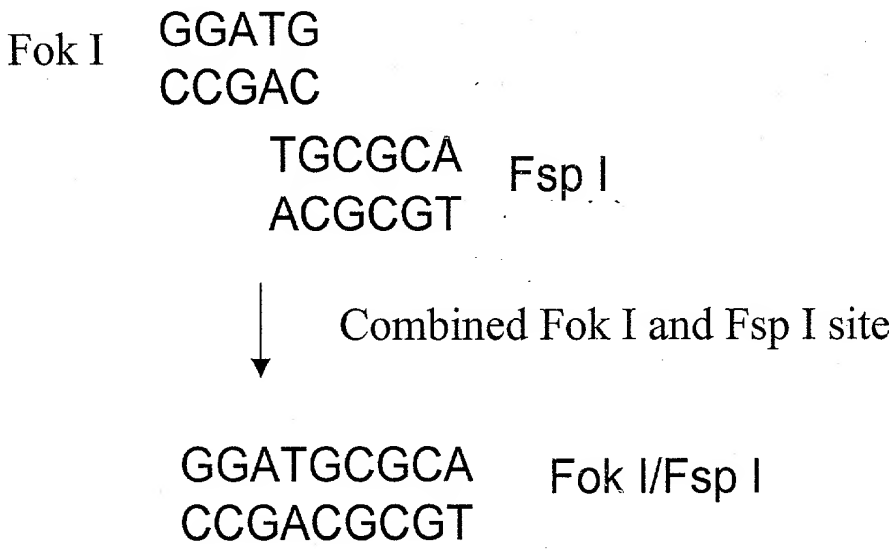

Fok I
GGATG
CCGAC
TGCGCA
ACGCGT
Fsp I
Combined Fok I and Fsp I site
GGATGCGCA
CCGACGCGT
Fok I/Fsp I

Figure 4

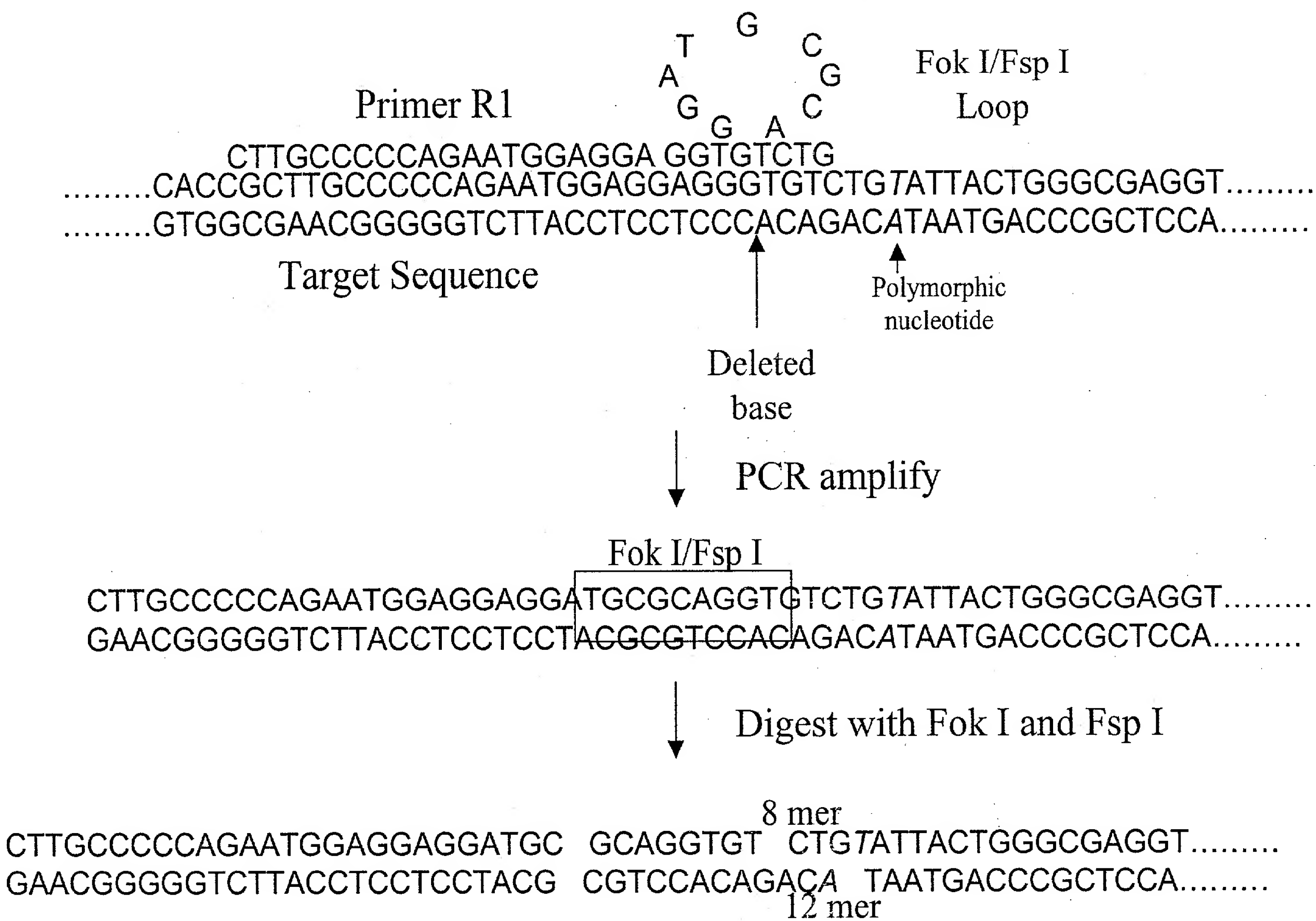

Primer R1
CTTGCCCCCAGAATGGAGGA GGTGTCTG
G A T G C G C A G G
Fok I/Fsp I
Loop
.........CACCGCTTGCCCCCAGAATGGAGGAGGGTGTCTGTATTACTGGGCGAGGT.........
.........GTGGCGAACGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCA.........
Target Sequence
Deleted base
Polymorphic nucleotide
PCR amplify
Fok I/Fsp I
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTGTATTACTGGGCGAGGT.........
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACATAATGACCCGCTCCA.........
Digest with Fok I and Fsp I
8 mer
CTTGCCCCCAGAATGGAGGAGGATGC GCAGGTGT CTGTATTACTGGGCGAGGT.........
GAACGGGGGTCTTACCTCCTCCTACG CGTCCACAGACA TAATGACCCGCTCCA.........
12 mer

Figure 5

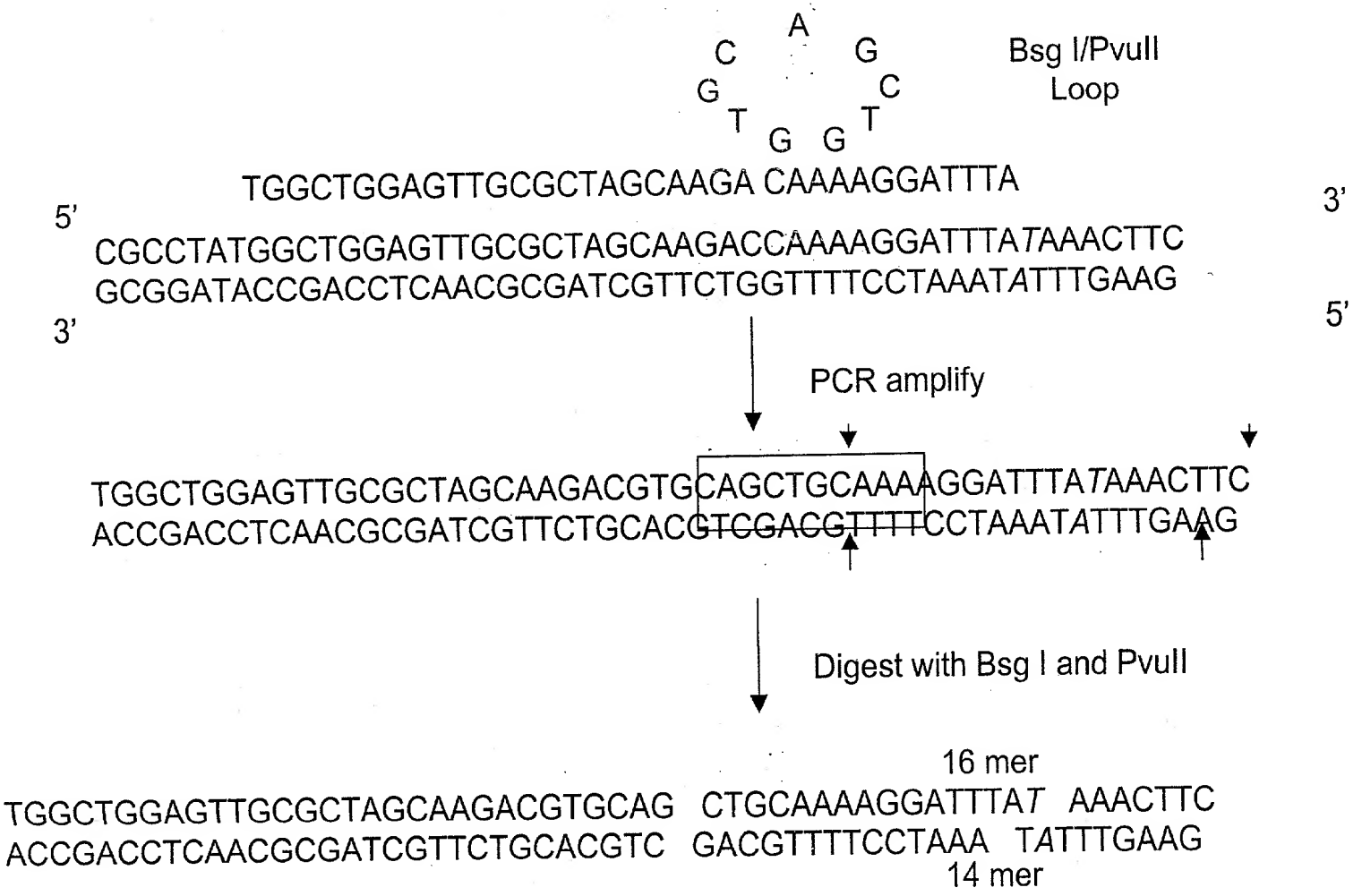

A
C
G
G
C
T
T
G G
Bsg I/PvuII
Loop
TGGCTGGAGTTGCGCTAGCAAGA CAAAAGGATTTA
5'
3'
CGCCTATGGCTGGAGTTGCGCTAGCAAGACCAAAAGGATTTATAAACTTC
GCGGATACCGACCTCAACGCGATCGTTCTGGTTTTCCTAAATATTTGAAG
3'
5'
PCR amplify
TGGCTGGAGTTGCGCTAGCAAGACGTGCAGCTGCAAAAGGATTTATAAACTTC
ACCGACCTCAACGCGATCGTTCTGCACGTCGACGTTTTCCTAAATATTTGAAG
Digest with Bsg I and PvuII
16 mer
TGGCTGGAGTTGCGCTAGCAAGACGTGCAG CTGCAAAAGGATTTAT AAACTTC
ACCGACCTCAACGCGATCGTTCTGCACGTC GACGTTTTCCTAAA TATTTGAAG
14 mer

Figure 6

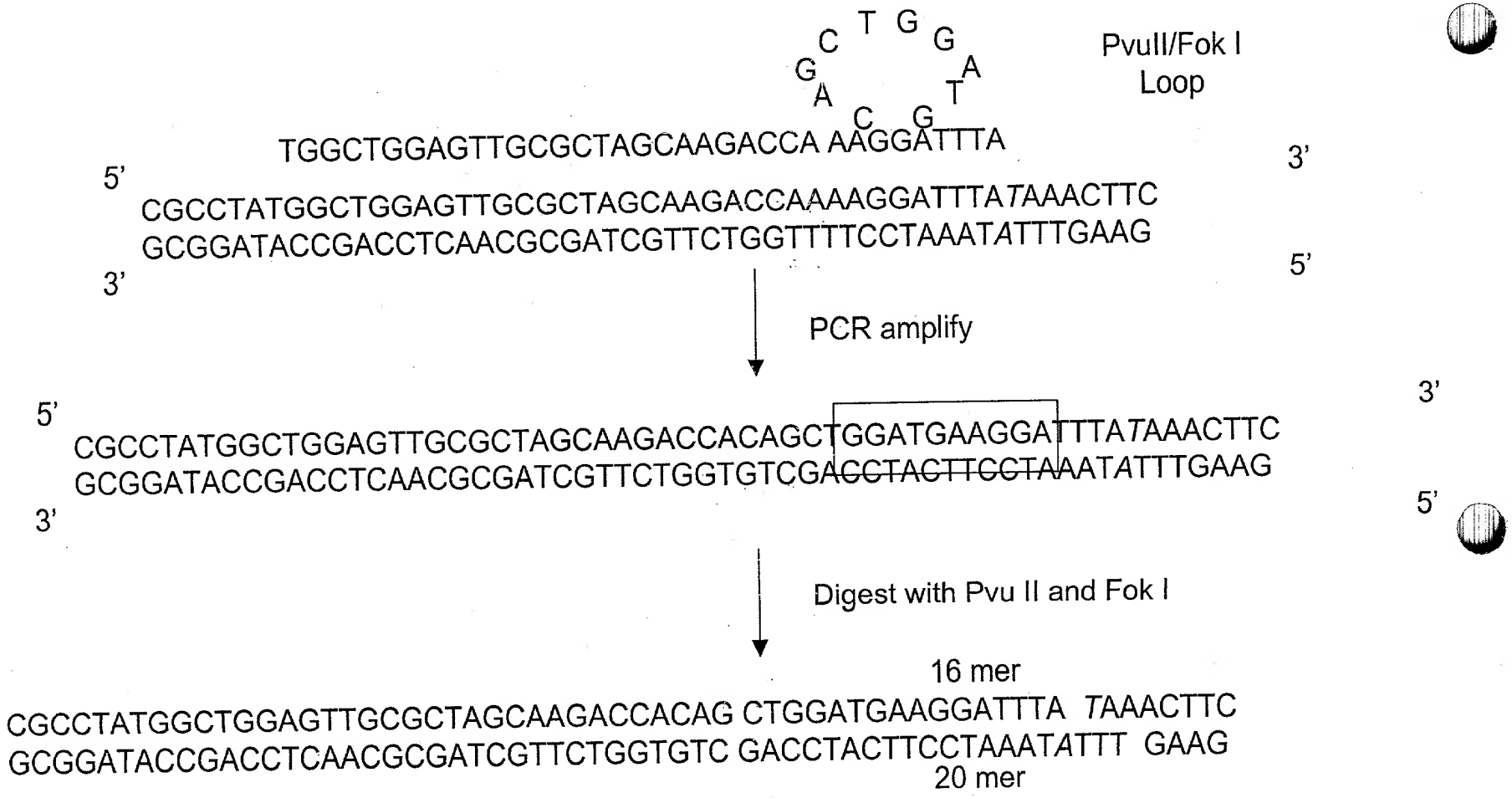

T G
C G
G A
A T
C G
PvuII/Fok I
Loop
TGGCTGGAGTTGCGCTAGCAAGACCA AAGGATTTA
5'
3'
CGCCTATGGCTGGAGTTGCGCTAGCAAGACCAAAAGGATTTATAAACTTC
GCGGATACCGACCTCAACGCGATCGTTCTGGTTTTCCTAAATATTTGAAG
3'
5'
PCR amplify
5'
3'
CGCCTATGGCTGGAGTTGCGCTAGCAAGACCACAGCTGGATGAAGGATTTATAAACTTC
GCGGATACCGACCTCAACGCGATCGTTCTGGTGTCGACCTACTTCCTAAATATTTGAAG
3'
5'
Digest with Pvu II and Fok I
16 mer
CGCCTATGGCTGGAGTTGCGCTAGCAAGACCACAG CTGGATGAAGGATTTA TAAACTTC
GCGGATACCGACCTCAACGCGATCGTTCTGGTGTC GACCTACTTCCTAAATATTT GAAG
20 mer

Figure 7

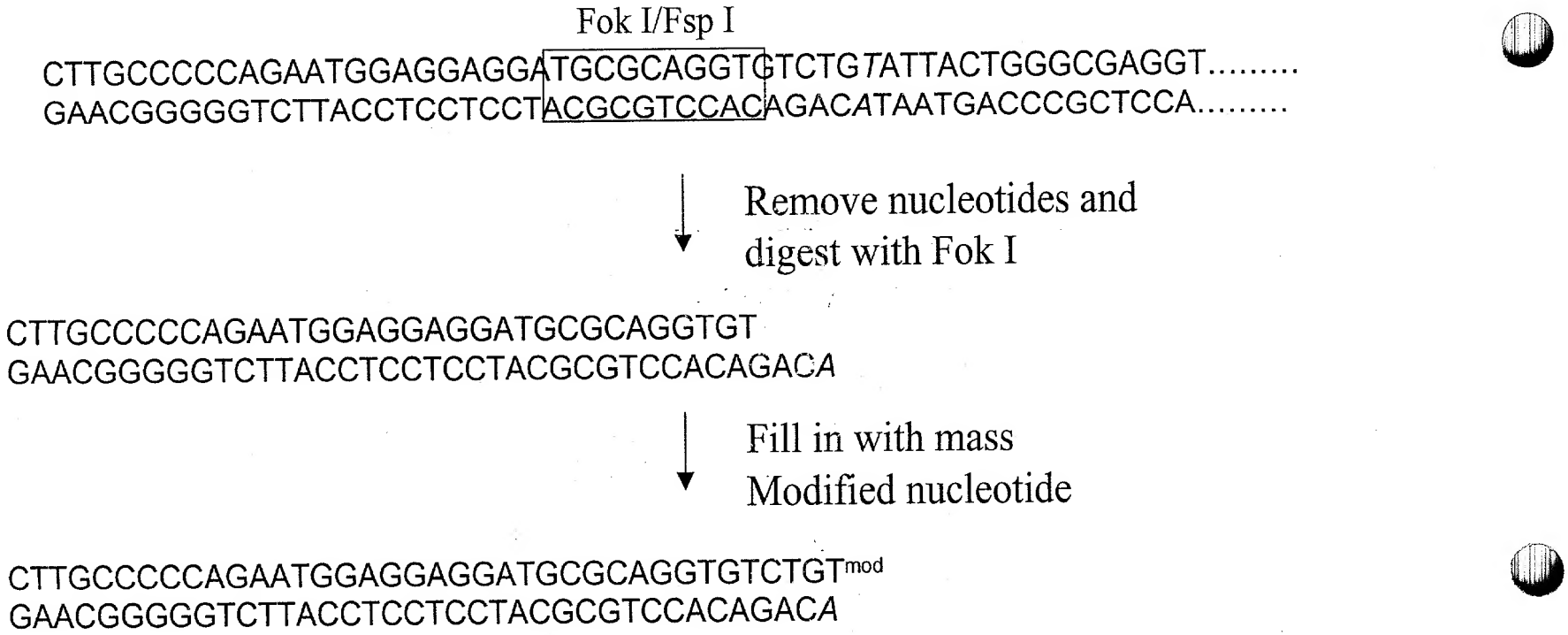

Fok I/Fsp I
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTGTATTACTGGGCGAGGT.....
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACATAATGACCCGCTCCA.....
Remove nucleotides and digest with Fok I
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGT
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACA
Fill in with mass Modified nucleotide
CTTGCCCCCAGAATGGAGGAGGATGCGCAGGTGTCTGTmod
GAACGGGGGTCTTACCTCCTCCTACGCGTCCACAGACA

Figure 8

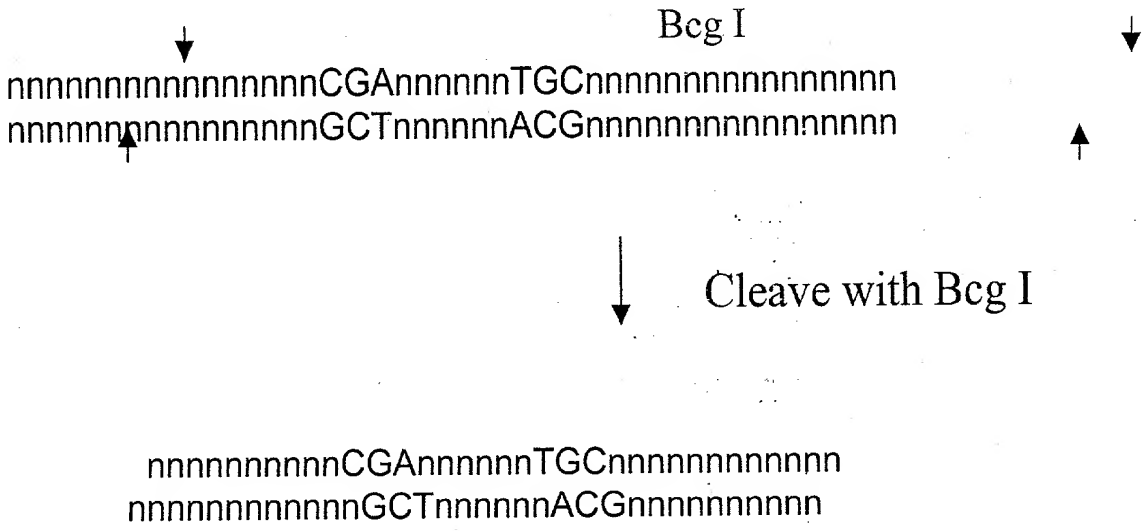

Bcg I
nnnnnnnnnnnnnnnnCGAnnnnnnTGCnnnnnnnnnnnnnnnn
nnnnnnnnnnnnnnnnGCTnnnnnnACGnnnnnnnnnnnnnnnn
Cleave with Bcg I
nnnnnnnnnnCGAnnnnnnTGCnnnnnnnnnnn
nnnnnnnnnnnnGCTnnnnnnACGnnnnnnnnnn

Figure 9

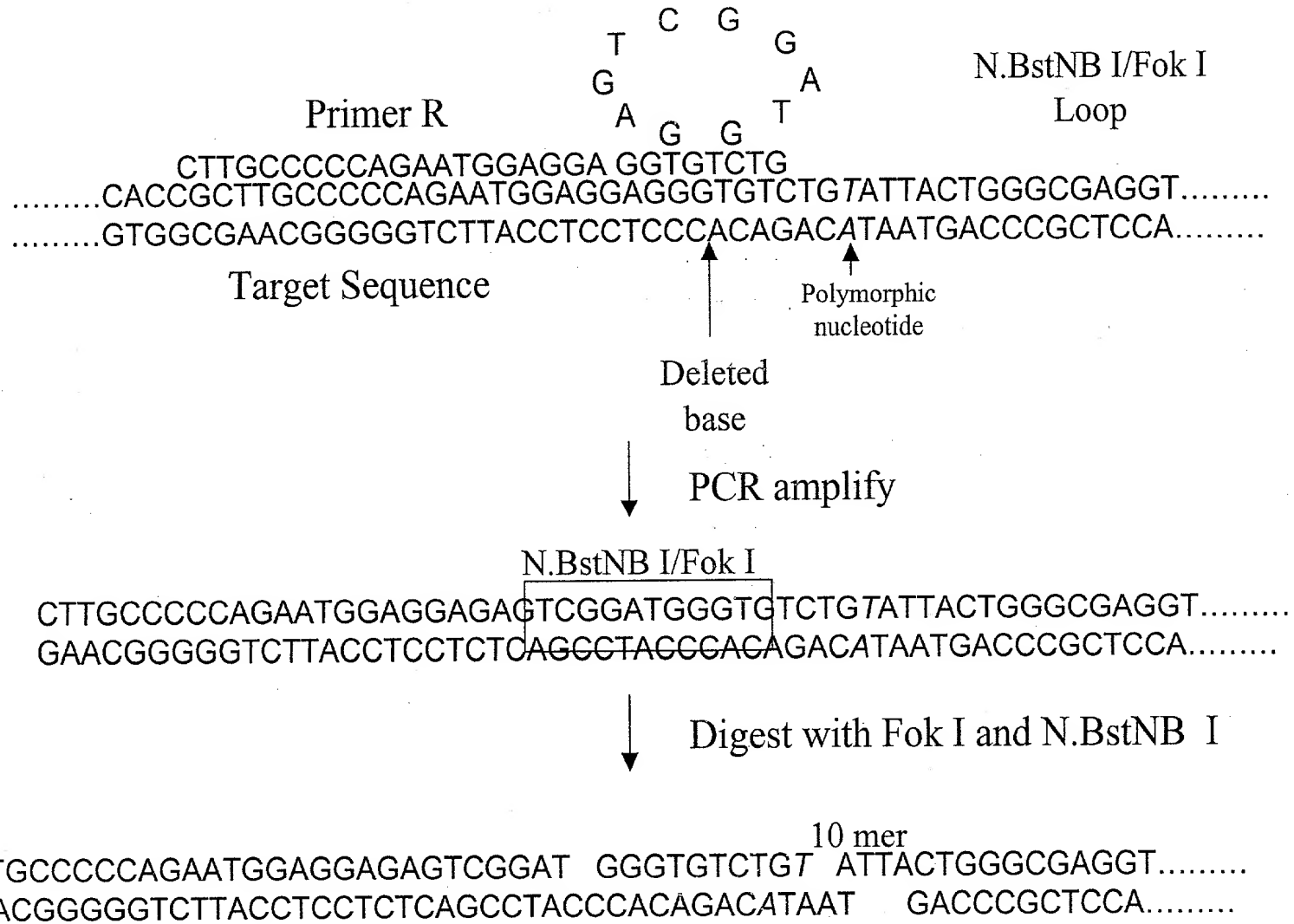

C G
T G
G A
A T
G G
Primer R
N.BstNB I/Fok I
Loop
CTTGCCCCCAGAATGGAGGA GGTGTCTG
.....CACCGCTTGCCCCCAGAATGGAGGAGGGTGTCTGTATTACTGGGCGAGGT.....
.....GTGGCGAACGGGGGTCTTACCTCCTCCCACAGACATAATGACCCGCTCCA.....
Target Sequence
Polymorphic nucleotide
Deleted base
PCR amplify
N.BstNB I/Fok I
CTTGCCCCCAGAATGGAGGAGAGTCGGATGGGTGTCTGTATTACTGGGCGAGGT.....
GAACGGGGGTCTTACCTCCTCTCAGCCTACCCACAGACATAATGACCCGCTCCA.....
Digest with Fok I and N.BstNB I
10 mer
CTTGCCCCCAGAATGGAGGAGAGTCGGAT GGGTGTCTGT ATTACTGGGCGAGGT.....
GAACGGGGGTCTTACCTCCTCTCAGCCTACCCACAGACATAAT GACCCGCTCCA.....

Figure 10

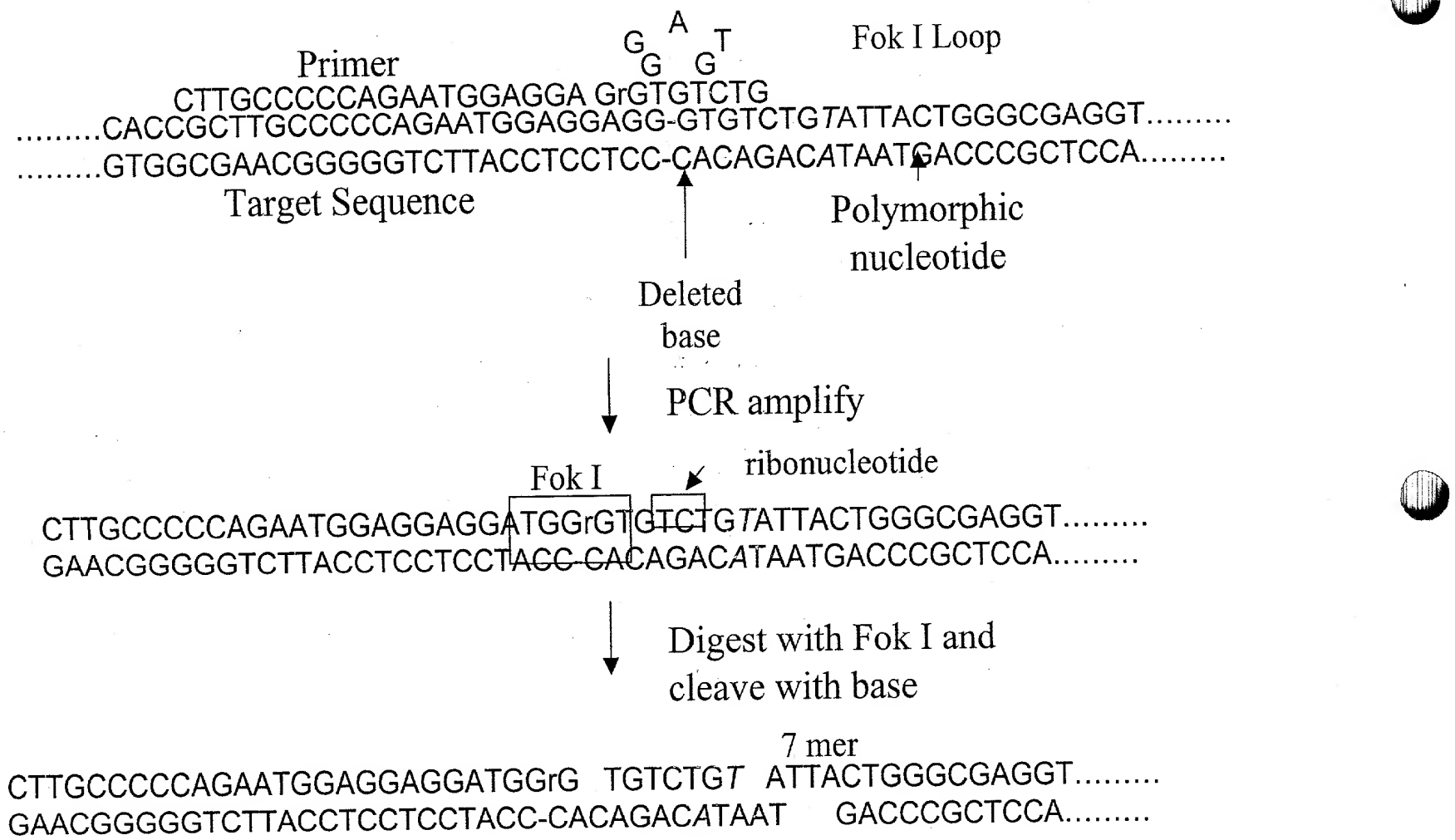

A
G G T
Fok I Loop
Primer
CTTGCCCCCAGAATGGAGGA GrGTGTCTG
.....CACCGCTTGCCCCCAGAATGGAGGAGG-GTGTCTGTATTACTGGGCGAGGT.....
.....GTGGCGAACGGGGGTCTTACCTCCTCC-CACAGACATAATGACCCGCTCCA.....
Target Sequence
Polymorphic nucleotide
Deleted base
PCR amplify
Fok I
ribonucleotide
CTTGCCCCCAGAATGGAGGAGGATGGrGTGTCTGTATTACTGGGCGAGGT.....
GAACGGGGGTCTTACCTCCTCCTACC-CACAGACATAATGACCCGCTCCA.....
Digest with Fok I and cleave with base
7 mer
CTTGCCCCCAGAATGGAGGAGGATGGrG TGTCTGT ATTACTGGGCGAGGT.....
GAACGGGGGTCTTACCTCCTCCTACC-CACAGACATAAT GACCCGCTCCA.....

Figure 11. Methods for haplotyping based on physical allele separation

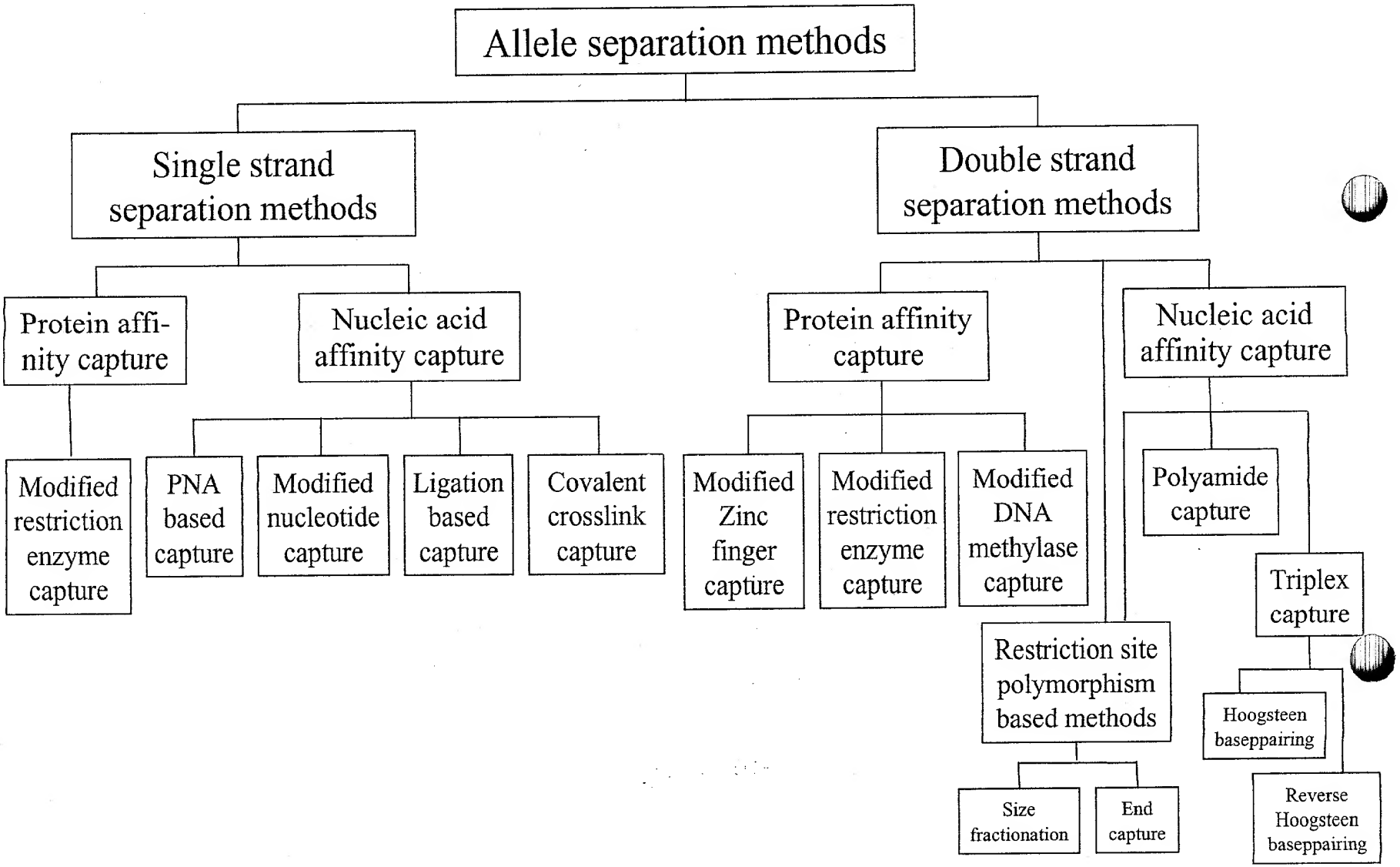
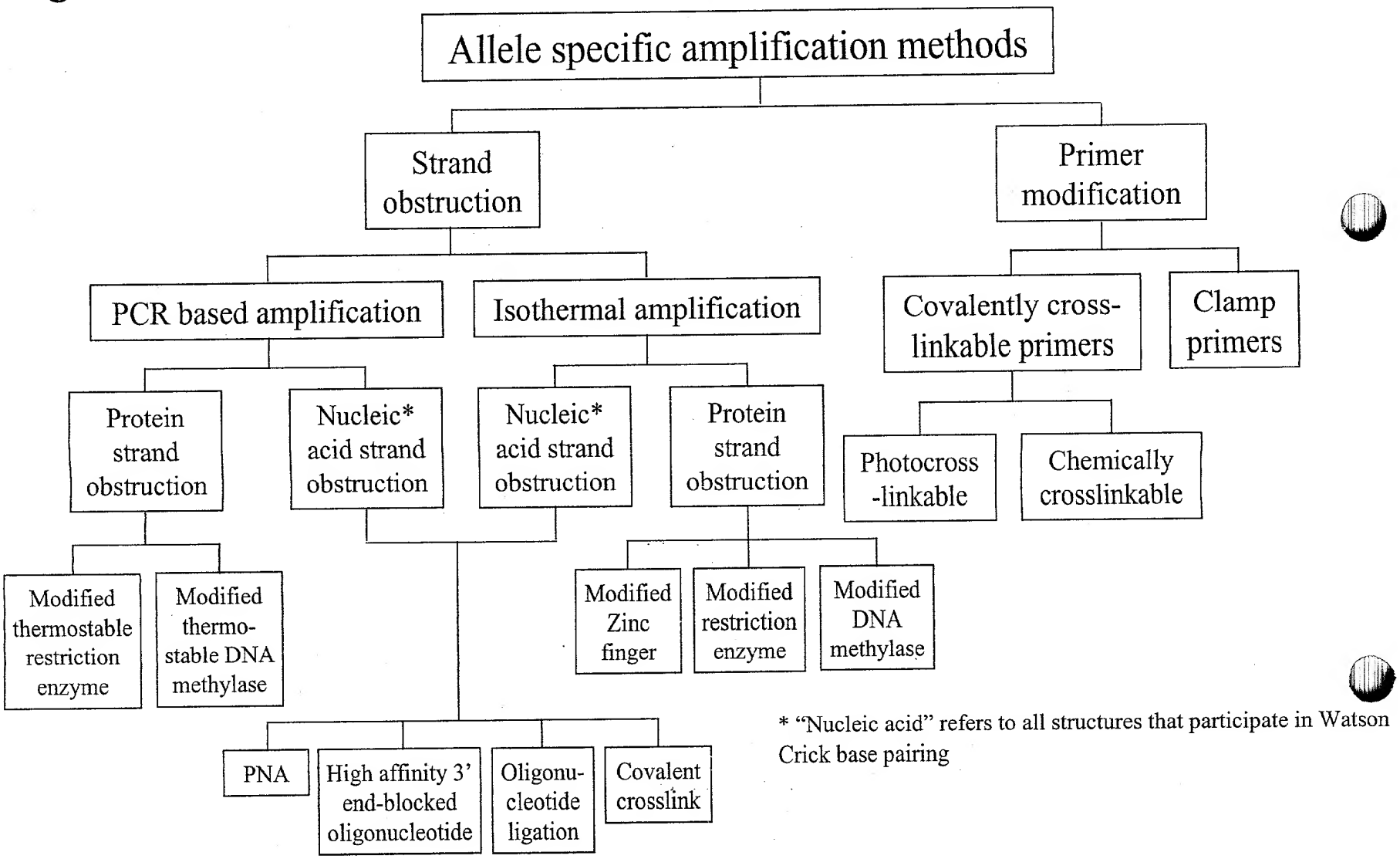


Figure 12. Methods for haplotyping based on allele specific amplification



* "Nucleic acid" refers to all structures that participate in Watson Crick base pairing

Figure 13. Methods for haplotyping based on allele specific restriction

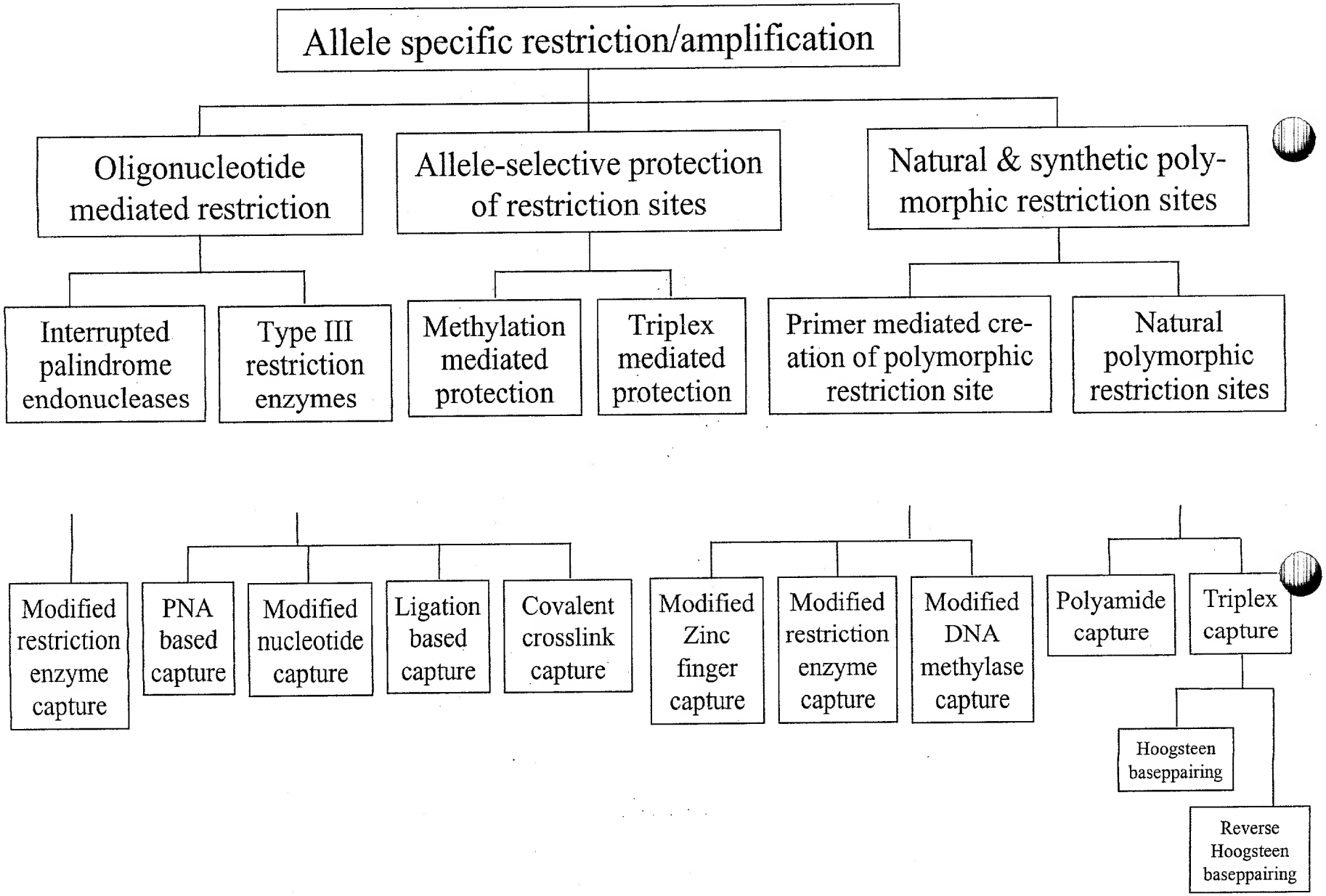


Figure 14: Hairpin PCR Primers

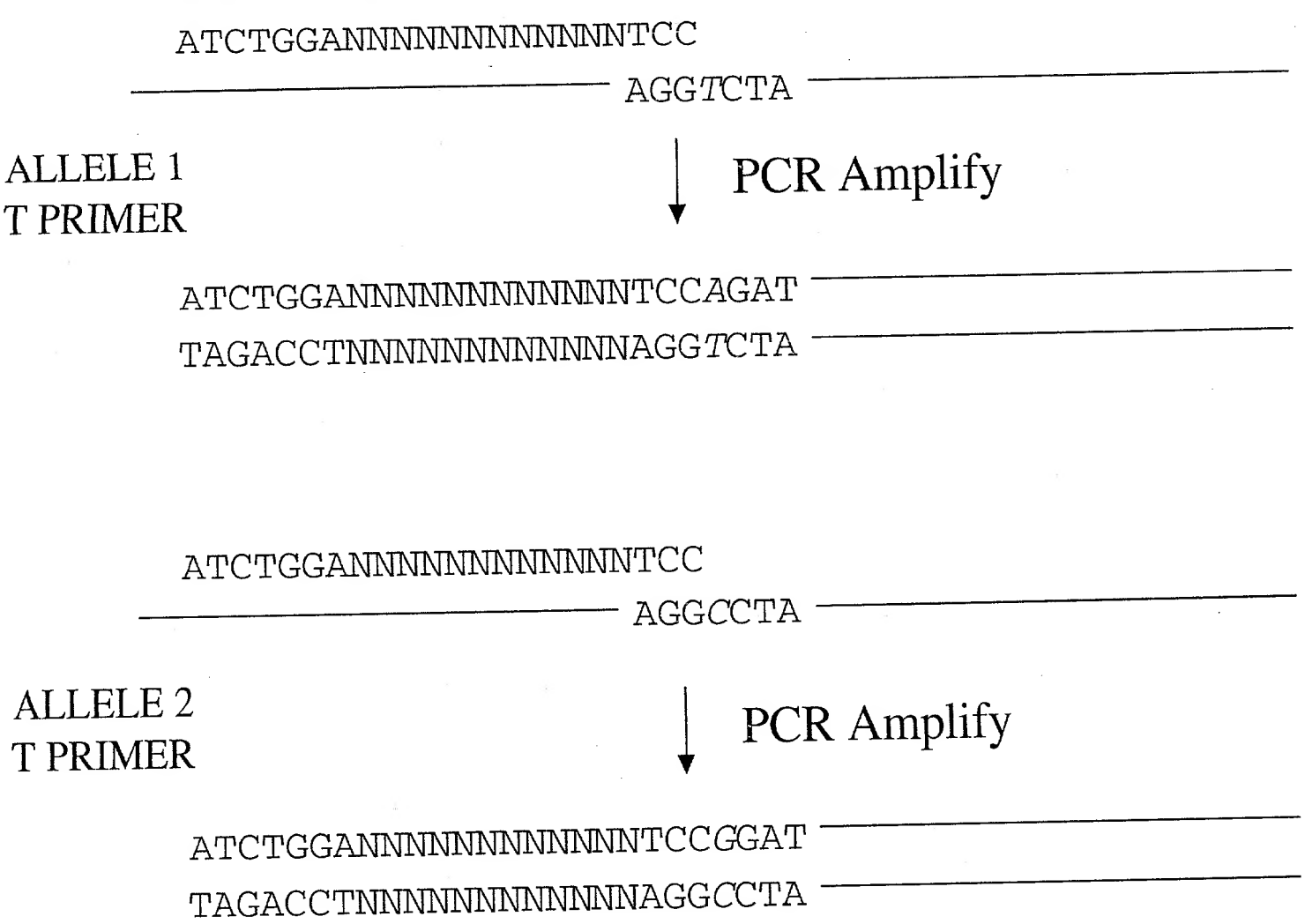


Figure 15: Hairpin PCR Primers

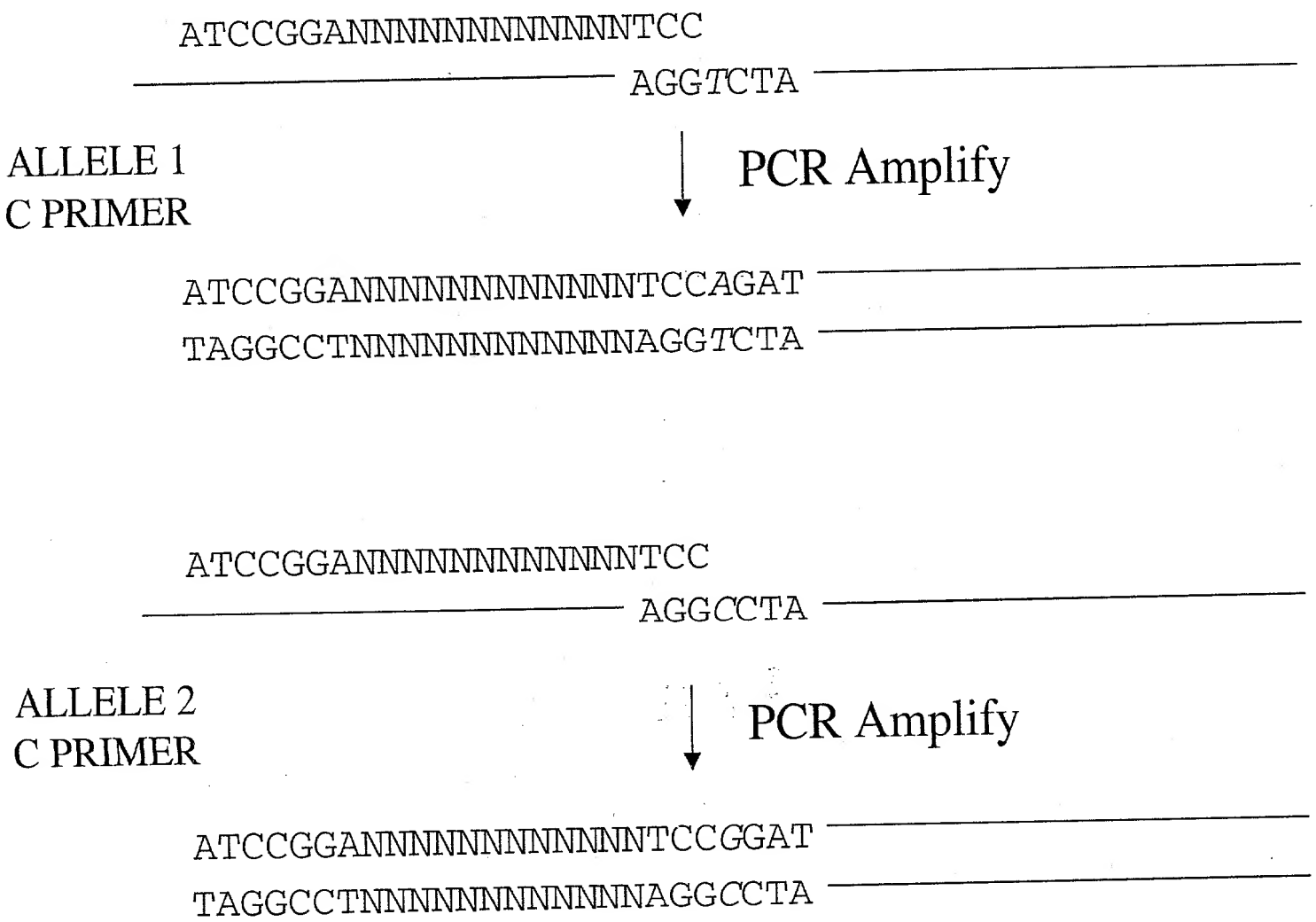


Figure 16: Hairpin PCR Primers

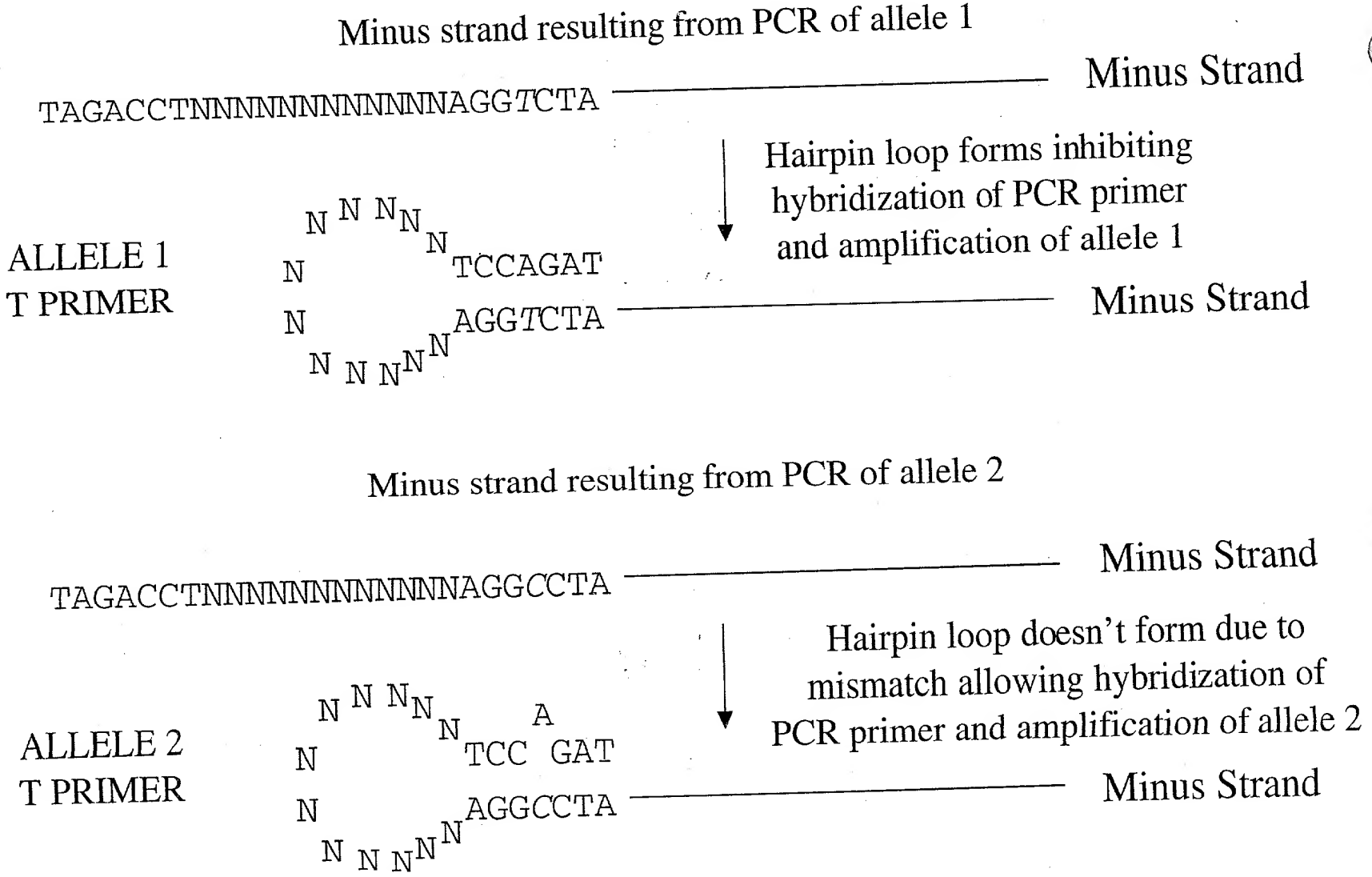


Figure 17: Hairpin PCR Primers

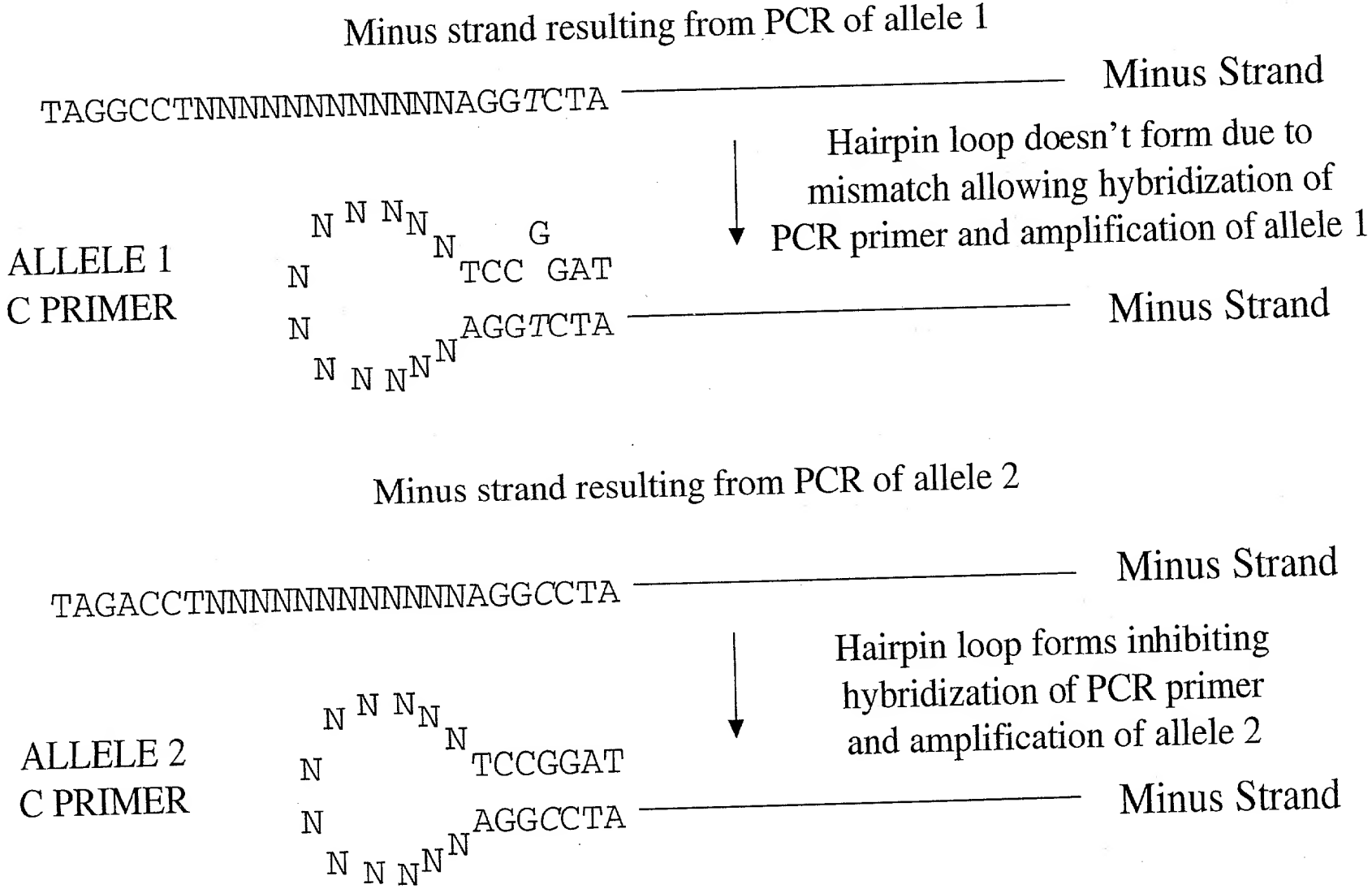


Figure 18

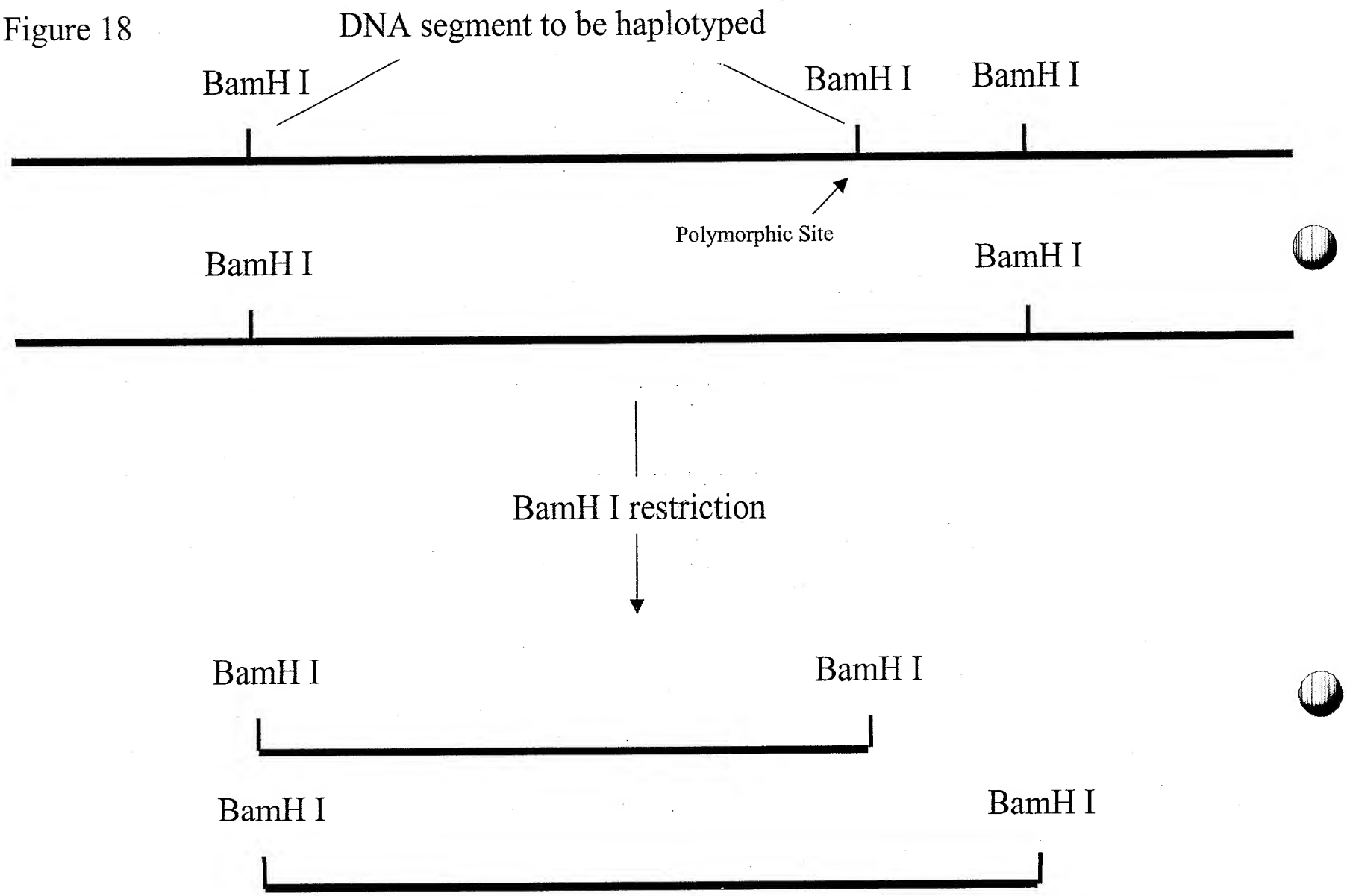

DNA segment to be haplotyped
BamH I
BamH I
BamH I
Polymorphic Site
BamH I
BamH I
BamH I restriction
BamH I
BamH I
BamH I
BamH I

Figure 19

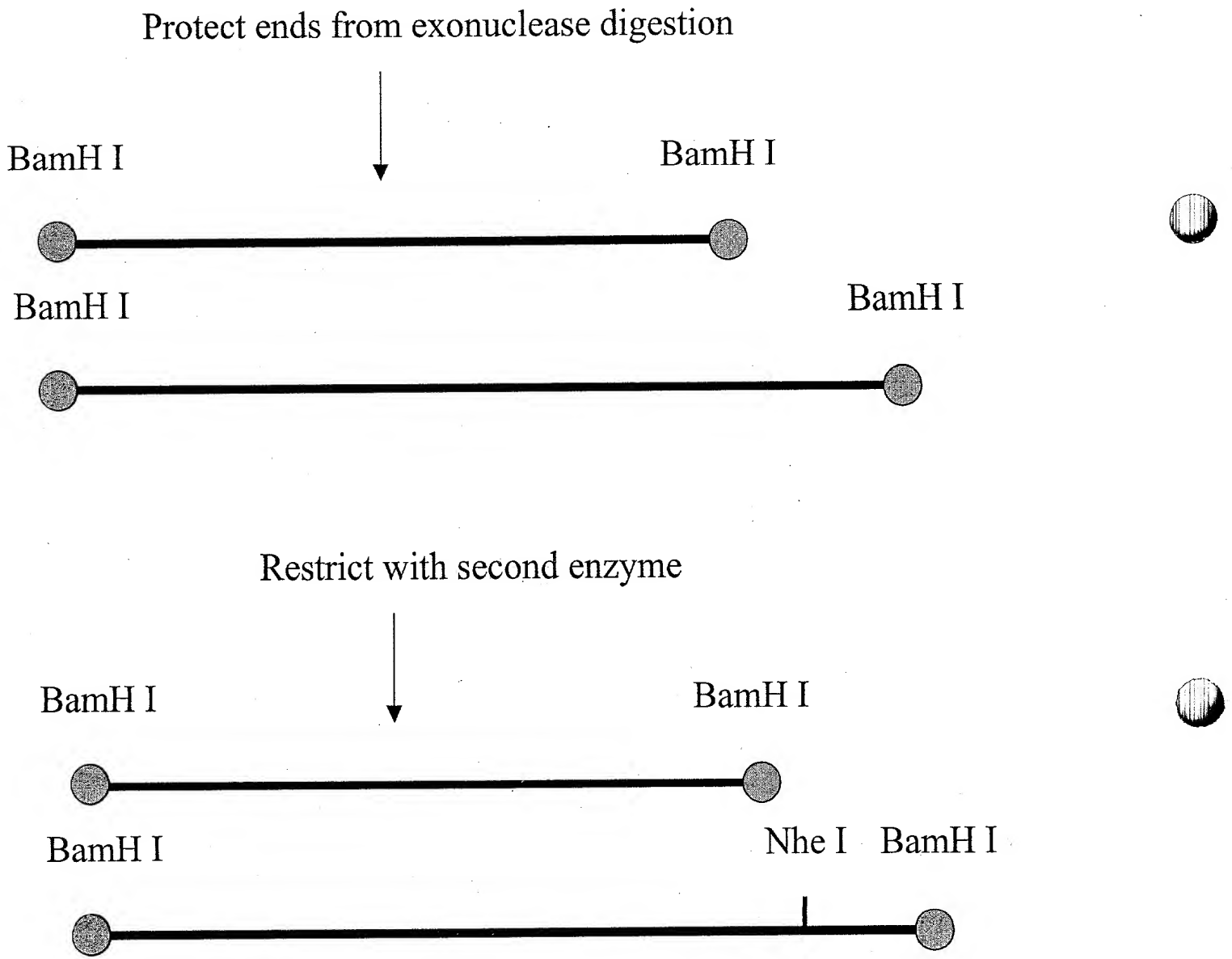

Protect ends from exonuclease digestion
BamH I
BamH I
BamH I
BamH I
Restrict with second enzyme
BamH I
BamH I
BamH I
Nhe I
BamH I

Figure 20

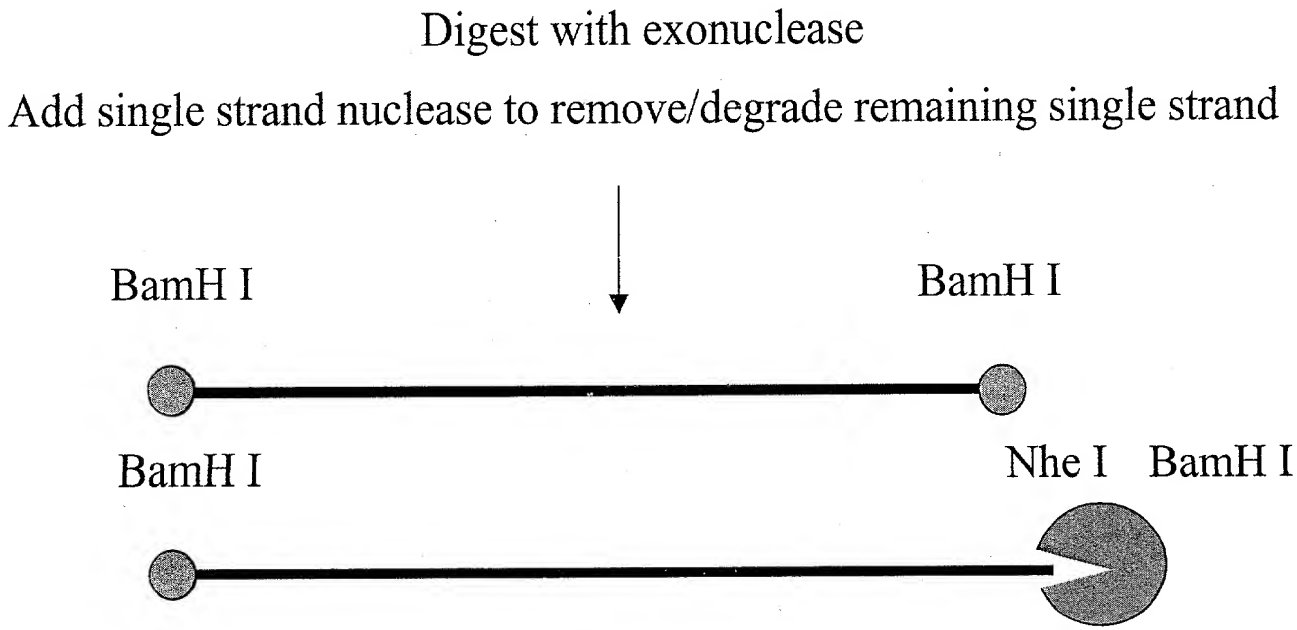

Digest with exonuclease
Add single strand nuclease to remove/degrade remaining single strand
BamH I
BamH I
BamH I
Nhe I
BamH I

Figure 21. Dihydropyrimidine dehydrogenase (DPD) polymorphisms used in haplotyping assay.

DPD polymorphisms

Base	Nucleotide	Amino Acid
186	T →	Cys
	C →	Arg
597	A →	Met
	G →	Val

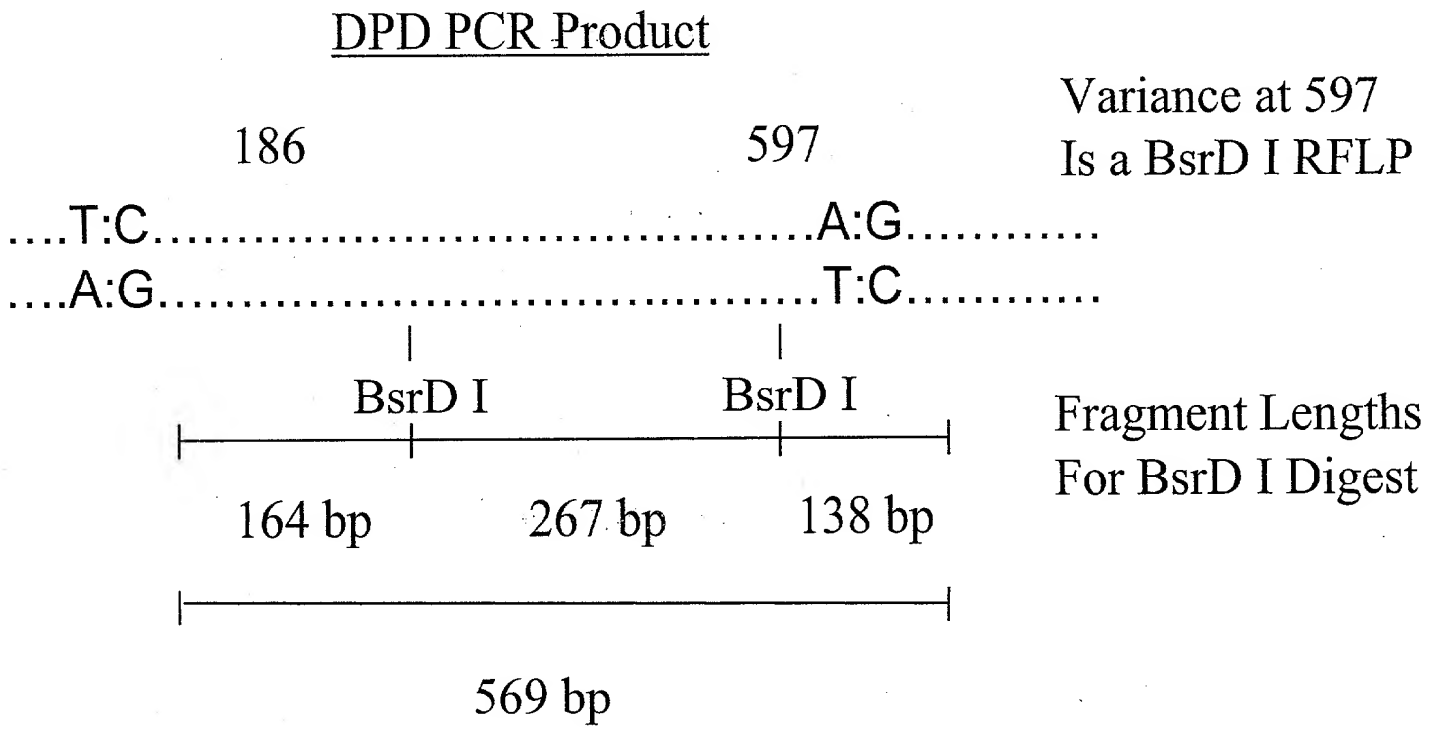


Figure 22. Allele Specific Primers for DPD

A.

DPD Primers	DPDASCF	5' acacagactcatgcaactctg	3'
	DPDASTF	5' acgcagactcatgcaactctg	3'
	DPDNSF	5' actcatgcaactctg	3'

B.

DPD Sequence

5' actcatgcaactctg[T or C]gttccacttcggccaagaa 3'

3' tgagtacgttgagac[A or G]caaggtgaagccggttctt 5'

Figure 23. PCR Amplification Using DPDNSF Primer

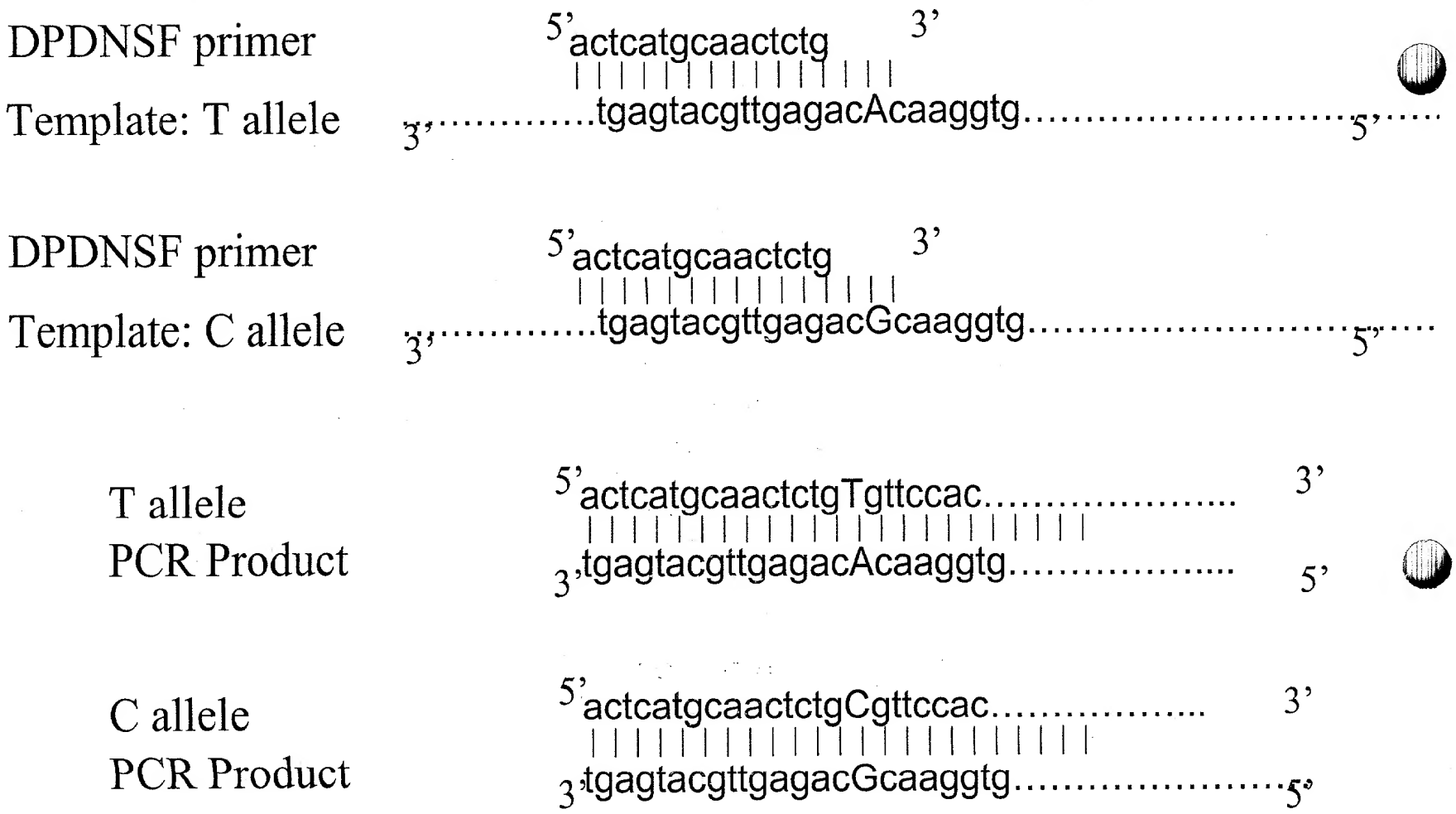


Figure 24. PCR Amplification Using DPDASTF Primer.

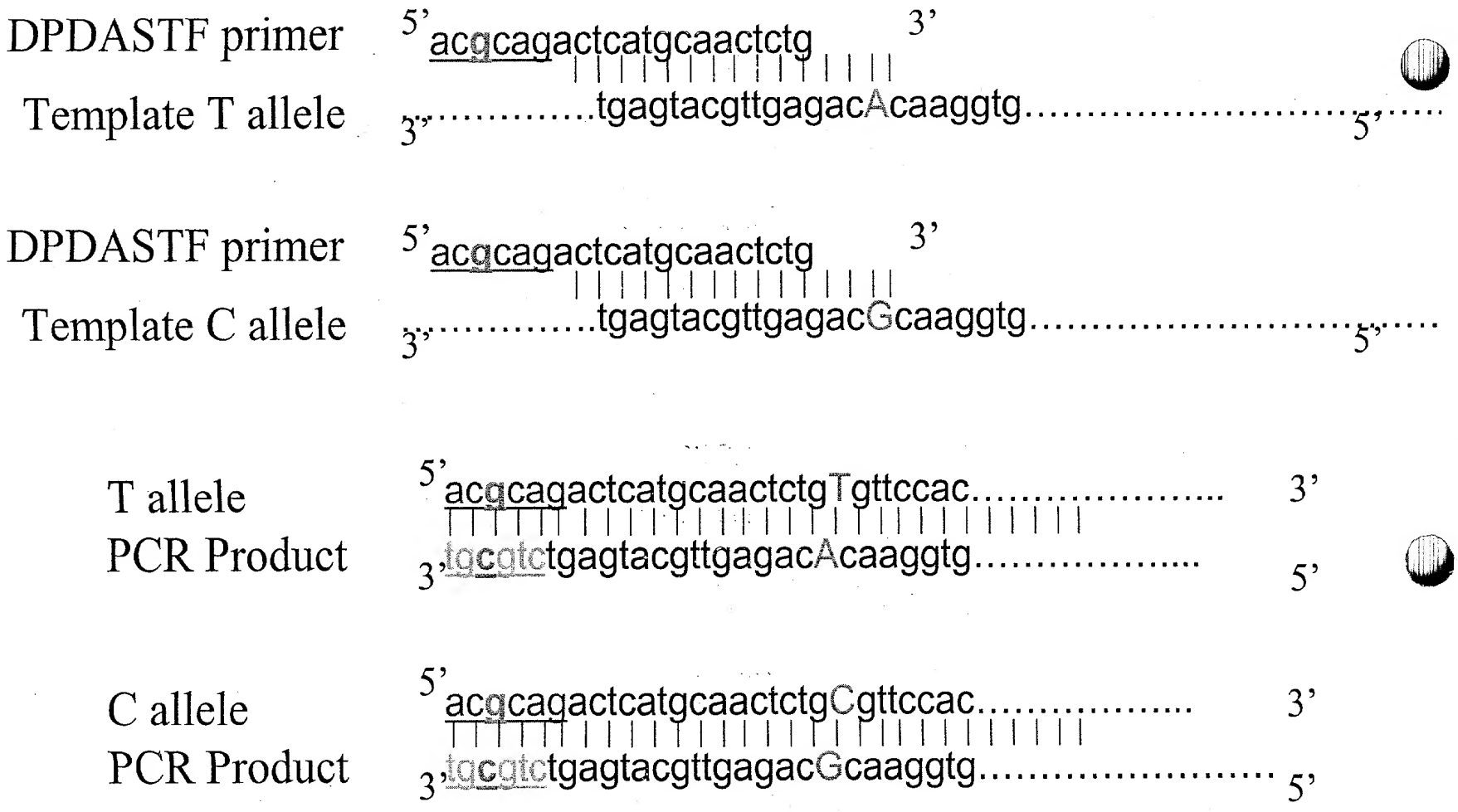


Figure 25. PCR Amplification Using DPDASCF Primer

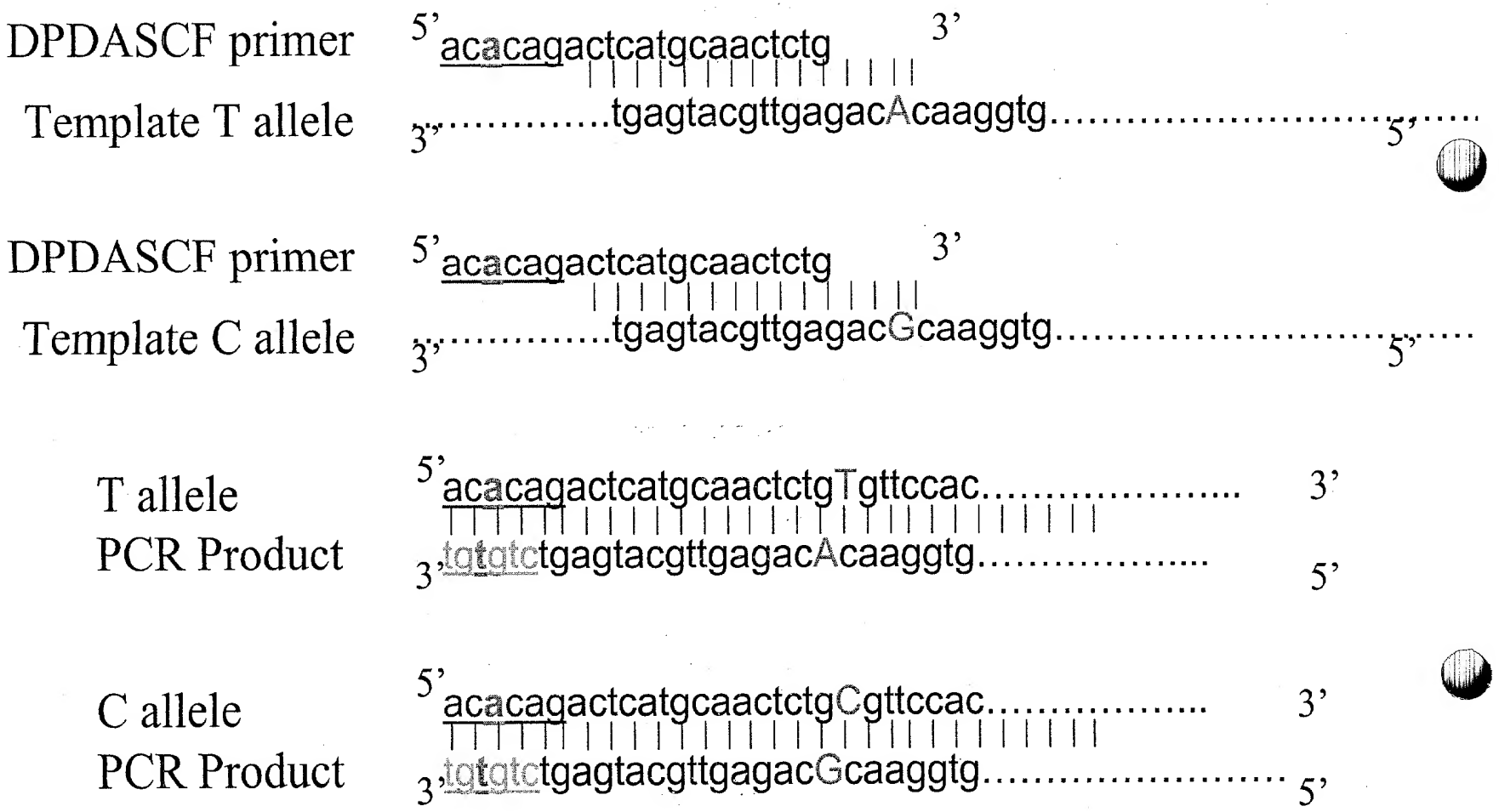
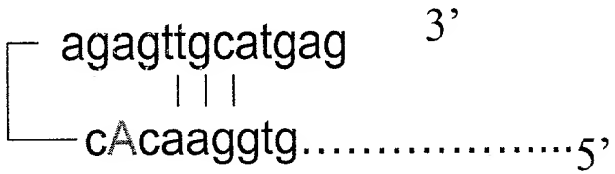


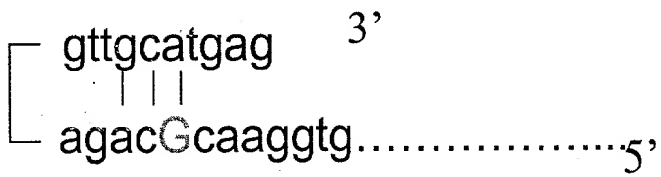
Figure 26. Hairpin Structures for PCR Products Generated Using DPDNSF Primer

Hairpin Structure T
Allele Reverse Strand



Not Stable

Hairpin Structure C
Allele Reverse Strand



Tm = 40°C

Figure 27. Hairpin Structures for PCR Products Generated Using DPDASCF Primer

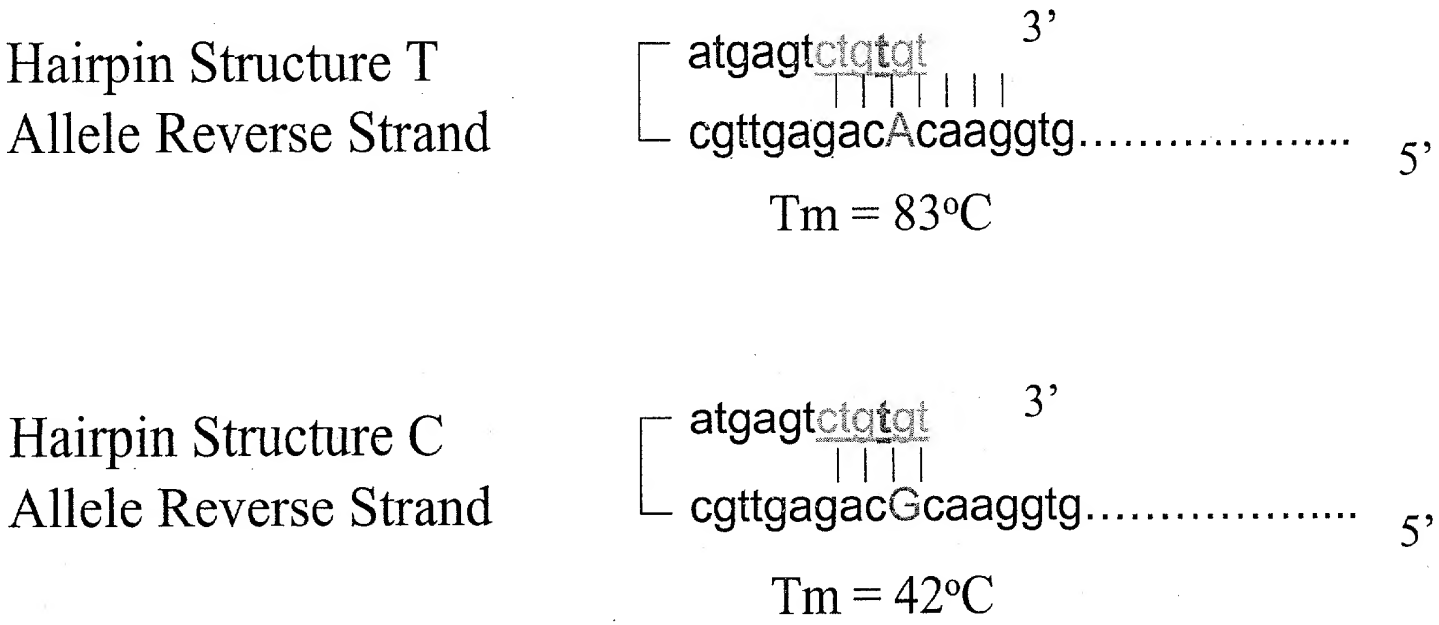


Figure 28. Hairpin Structures for PCR Products Generated Using DPDASTF Primer

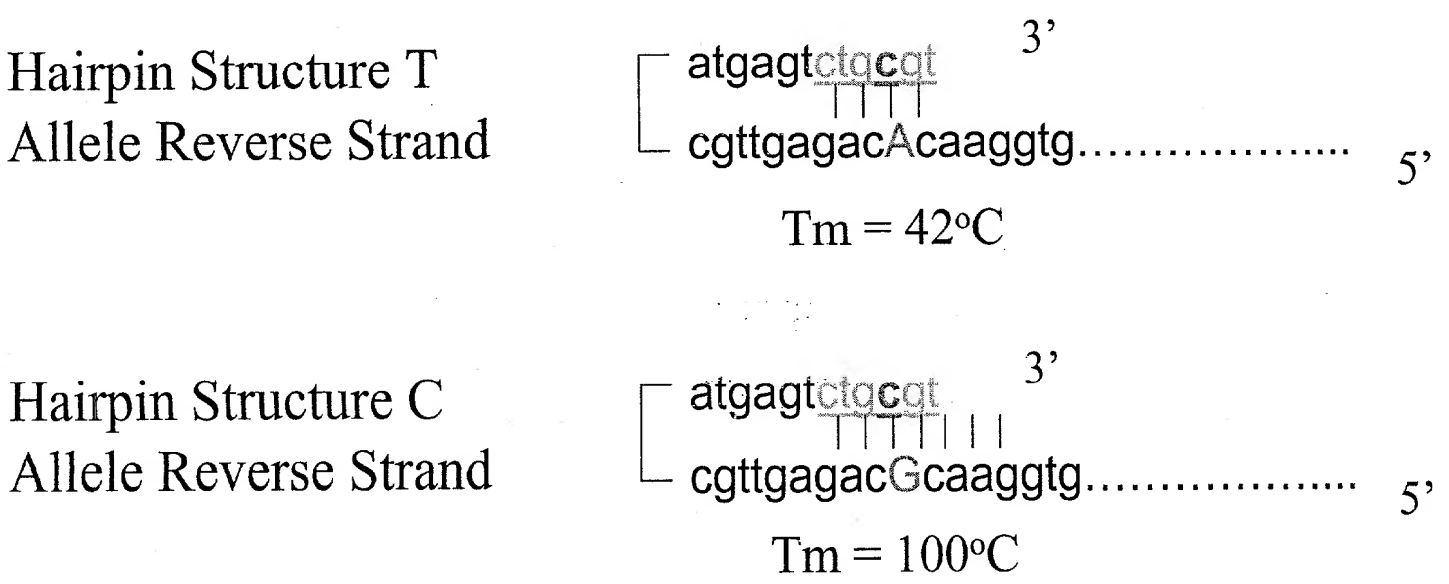


Figure 29. Non-Allele Specific Amplification Using DPDNSF Primer.

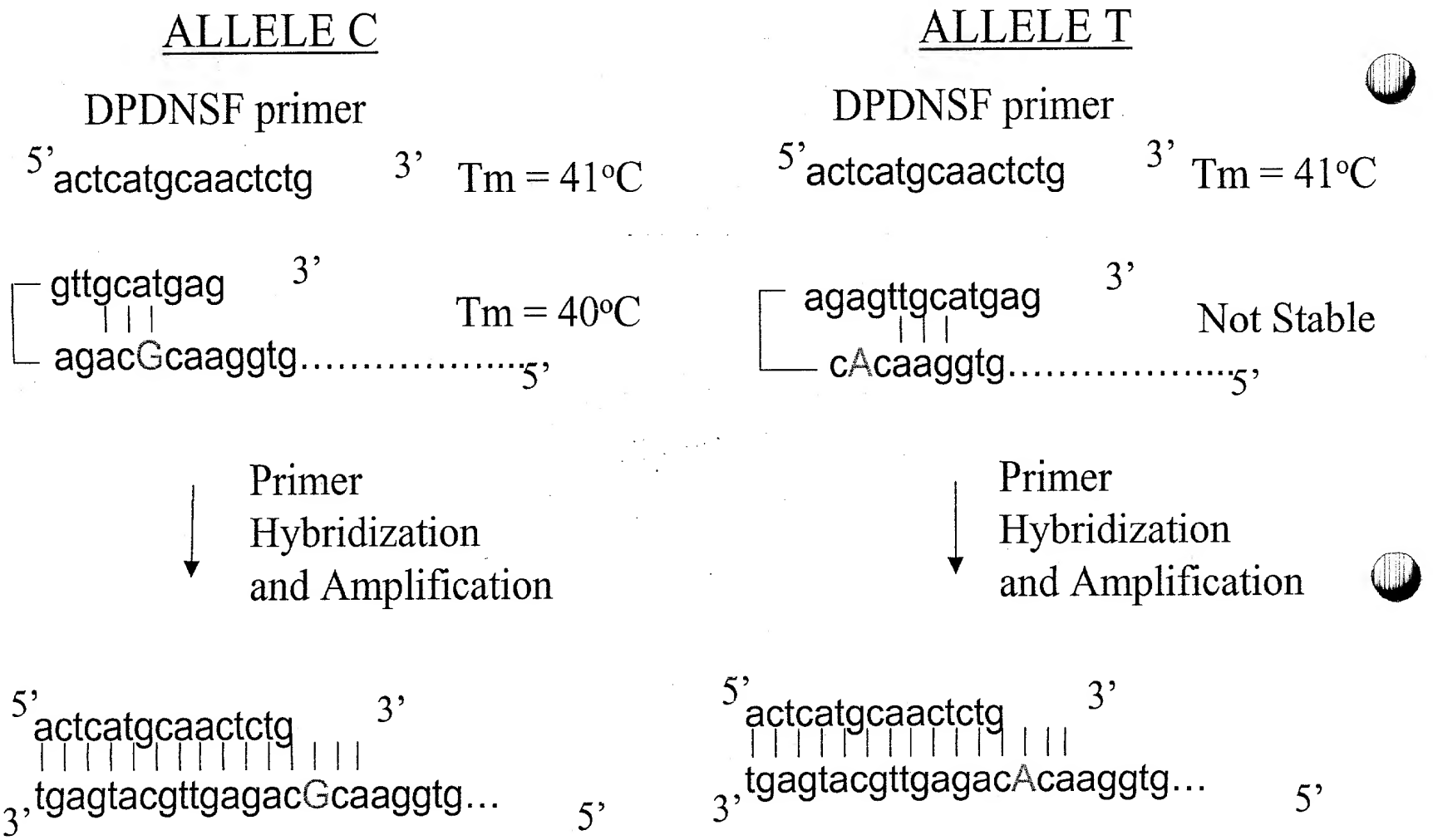


Figure 30. Allele Specific Amplification Using DPDASCF Primer

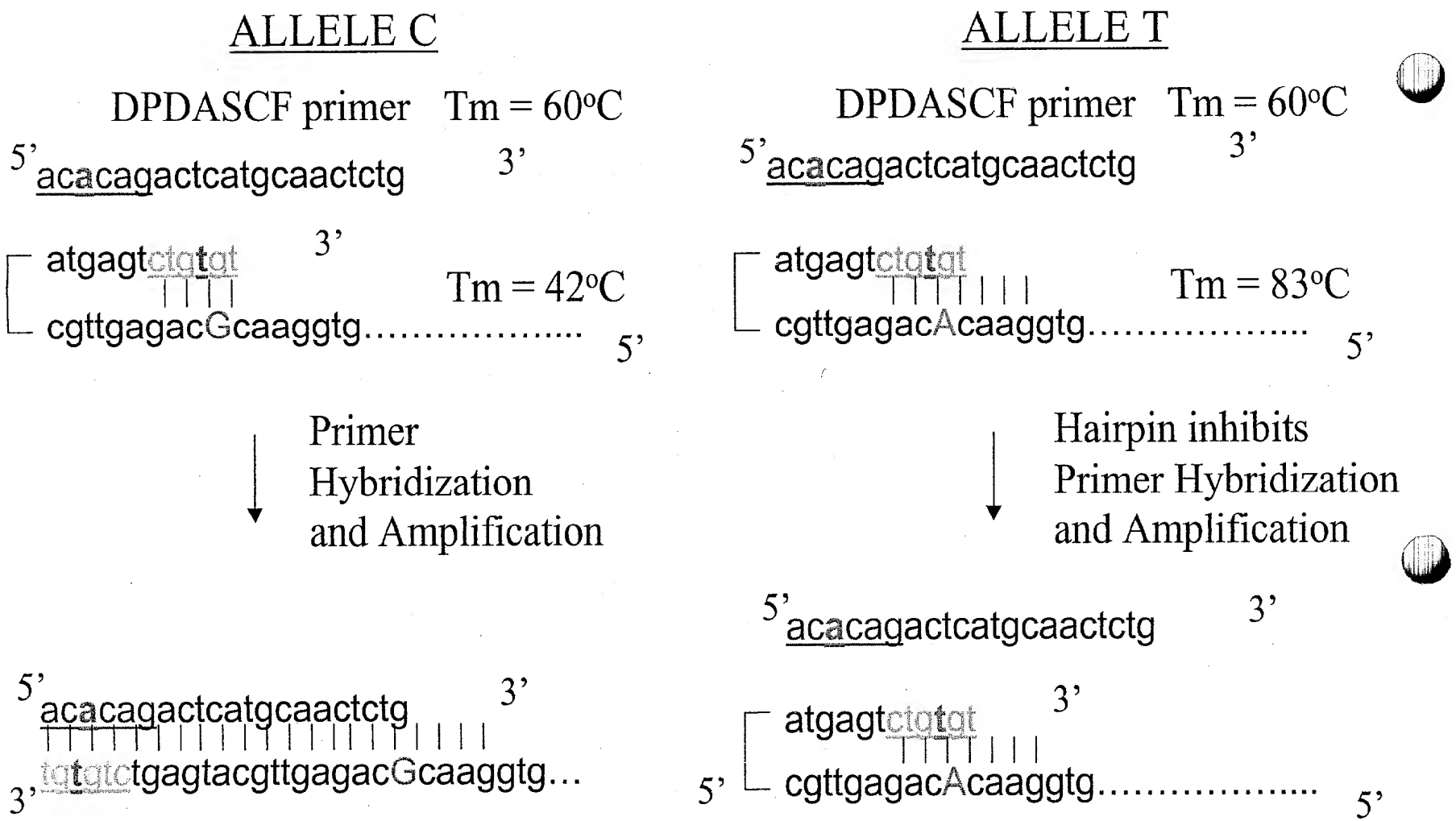


Figure 31. Allele Specific Amplification Using DPDASTF Primer

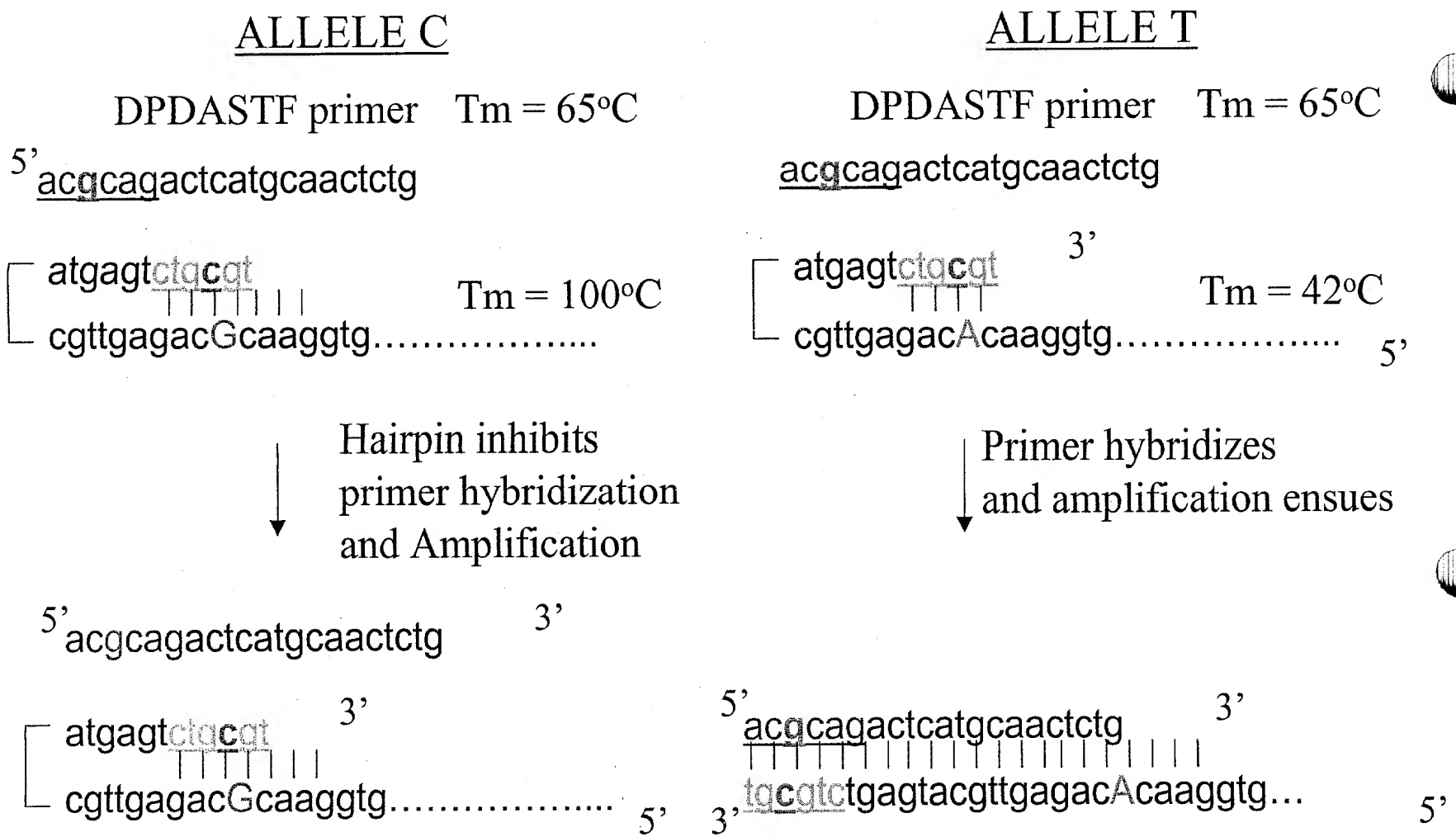


Figure 32. Allele Specific Amplification of a Heterozygous Sample with Haplotype T^{186},A^{597} and C^{186},G^{597}

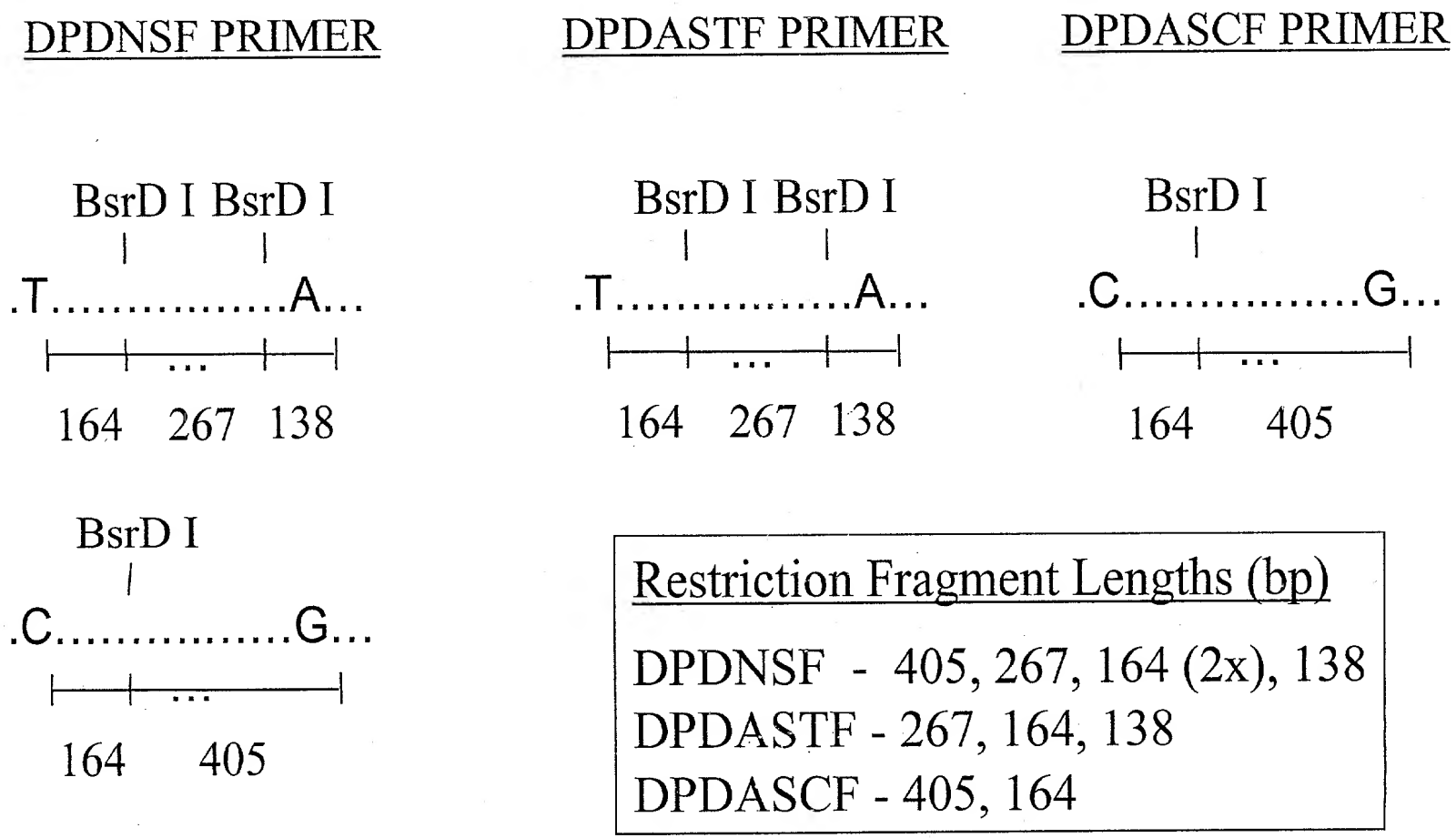


Figure 33. BsrD I Digest of Allele Specific PCR Products.

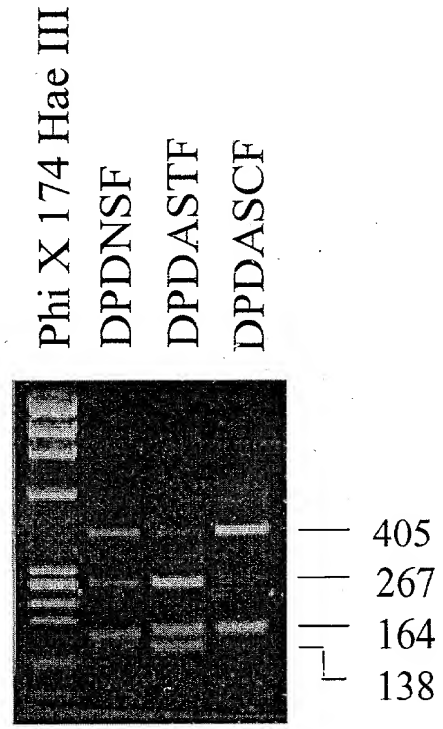


Figure 34

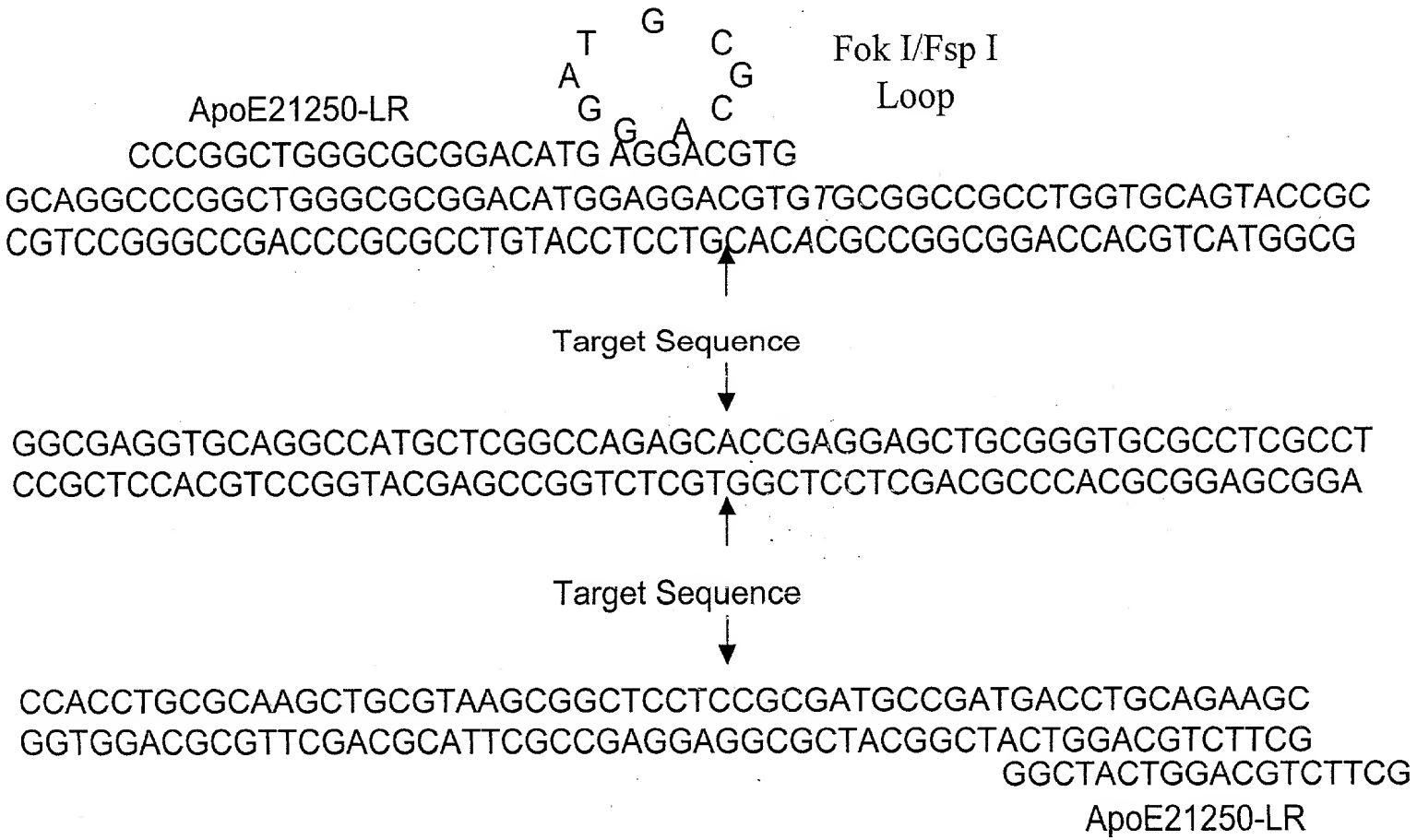
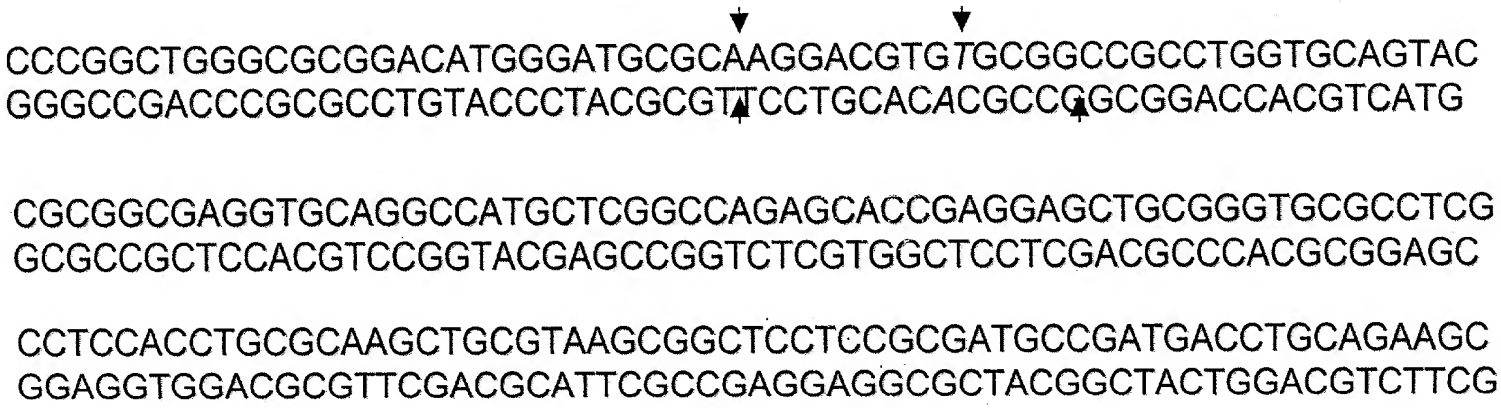

G
T
C
Fok I/Fsp I
A
G
Loop
ApoE21250-LR
G
C
G
A
CCCGGCTGGGCGCGGACATG AGGACGTG
GCAGGCCCGGCTGGGCGCGGACATGGAGGACGTGTGCGGCCGCCTGGTGCAGTACCGC
CGTCCGGGCCGACCCGCGCCTGTACCTCCTGCACACGCCGGCGGACCACGTCATGGCG
Target Sequence
GGCGAGGTGCAGGCCATGCTCGGCCAGAGCACCGAGGAGCTGCGGGTGCGCCTCGCCT
CCGCTCCACGTCCGGTACGAGCCGGTCTCGTGGCTCCTCGACGCCCACGCGGAGCGGA
Target Sequence
CCACCTGCGCAAGCTGCGTAAGCGGCTCCTCCGCGATGCCGATGACCTGCAGAAGC
GGTGGACGCGTTCGACGCATTCGCCGAGGAGGCGCTACGGCTACTGGACGTCTTCG
GGCTACTGGACGTCTTCG
ApoE21250-LR

Figure 35

T Allele Amplicon



C Allele Amplicon